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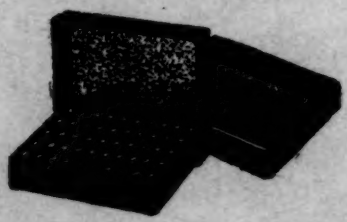

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The Journal of Laboratory and Clinical Medicine

VOL. III. ST. LOUIS, FEBRUARY, 1918 No. 5

ORIGINAL ARTICLES

METHODS OF CONTROL OF THE CLOTHES LOUSE [PEDICULUS HUMANUS (VESTIMENTI)]*

BY WILLIAM MOORE, ST. PAUL, MINN.

INTRODUCTION

LATE in April, 1917, at the suggestion of A. D. Hirschfelder and with the approval of the local branch of the National Research Council, I undertook a study of the methods of control of the clothes louse.† Peacock[1] has very clearly defined the problem, and from his description of conditions, there appear four possible methods which might be adopted for the destruction of the lice. These may be briefly summarized as; first, the destruction of the lice by the vapor of a volatile organic compound worn by the soldier as a sachet; second, the use of louse powders; third, impregnation of the underwear with an insecticide; fourth, the destruction by heat or fumigation of both lice and eggs in the garments, while the soldier is bathing.

DESTRUCTION BY MEANS OF SACHETS

Naphthalene,[2] camphor,[3] sulphur,[4] paradichlorbenzene,[5] and various other chemicals[6] have been recommended to be worn in small bags about the neck or the waist of the soldier. The principle of the sachet is the production of a poisonous vapor or gas in the space between the body of the soldier and his outer garments. Actual trials, of these and many other chemicals of about the same volatility, in our experiments show that lice will continue to live under such conditions and will even lay eggs. In some other investigations[7, 8] I have shown that the toxicity of volatile organic compounds to insects increases as the boiling

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†I wish to express my thanks to S. A. Graham and Miss Anna Wentz for assistance in the care and rearing of the lice used in the experiments.

point rises and the volatility decreases. Compounds with a boiling point higher than 225° C. in general are so slightly volatile that not sufficient vapor is produced to kill the insects within a reasonable period of time. Theoretically, therefore, such compounds as naphthalene, camphor, and paradichlorbenzene should be the best insecticides for use in the destruction of lice. The failure of these compounds must, therefore, be due either to the lack of diffusion or the rapidity with which the vapor escapes. In large glass containers where there is no opportunity of escape, the vapor diffuses in a few hours sufficiently to kill lice in all parts of the flask. It appears, therefore, that the ineffectiveness of these compounds must be due to the rapidity with which their vapors escape through the clothing. The following experiments were conducted to determine the accuracy of this point.

Five-gram lots of the compounds to be tested were weighed out in watch glasses and placed in wide-mouthed jars lying on their sides. The openings of the jars were covered with different types of cotton, wool, and silk underwear, woolen and khaki clothes, and combinations of these to as many as three or four thicknesses. A control jar was left open while another control was closed with a glass top. From a comparison of the amount of evaporation from these jars, it is possible to arrive at an estimation of the amount of diffusion through the different types of clothing. In the case of the jar closed with a glass top, the air soon became saturated with the vapor after which no further evaporation occurred. This amount was so small in the case of camphor, naphthalene, and paradichlorbenzene, that no difference in weight could be detected in the com-

TABLE I

EVAPORATION OF VOLATILE COMPOUNDS FROM JARS CLOSED WITH DIFFERENT KINDS OF CLOTH

NAPHTHALENE	
Check,	100%
Light cotton gauze underwear,	66%
Medium cotton and wool underwear,	88%
Medium cotton underwear,	66%
Medium silk and cotton underwear,	66%
Medium silk underwear,	88%
Heavy cotton fleece-lined underwear,	88%
Heavy cotton and wool underwear,	85%
Blue flannel,	77%
Double blue flannel,	44%
Muslin underwear, woolen shirt, khaki coat,	50%
PARADICHLORBENZENE	
Check,	100%
Medium wool and cotton underwear,	62%
Fleece-lined cotton underwear,	55%
Light cotton gauze underwear,	60%
XYLENE	
Check,	100%
Fleece-lined cotton underwear,	46%
Light gauze cotton underwear,	50%
Medium silk underwear,	50%
Medium wool and cotton underwear,	42%
Heavy cotton underwear,	46%
Muslin underwear, woolen shirt, khaki coat	43%

pound. Evaporation from the open jar was considered as 100 per cent and on this basis is was found that naphthalene diffusing through different types of clothing varied from 44 to 88 per cent, and paradichlorbenzene, from 55 to 62 per cent (see Table I).

From these results it is apparent that not more than 15 to 50 per cent of a saturated atmosphere of these compounds could be retained under the soldier's uniform; or, in other words, the vapor escapes the clothing almost as rapidly as the compound evaporates. Such a low percentage of saturation is not sufficiently high to result in effective control of the lice. Similar experiments conducted with a more volatile compound such as xylene mixed with fuller's earth showed a leakage of from 40 to 45 per cent. Fifty per cent of a saturated atmosphere of xylene could thus be maintained, and this quantity is sufficient to produce the death of lice. In actual experience, where the lice were not confined in a vial, it was found that they were merely stupified and lodged in the lower portions of the trouser legs, where, not sufficient vapor being present, they revived. To overcome this difficulty sachets might be attached about the knees on both legs, but when the large quantity of xylene which is necessary to maintain the desired degree of saturation is considered, it is at once apparent that such treatment is eliminated by its expense.

As a summary of the results of the work with sachets, it might be stated that compounds such as naphthalene, camphor, or paradichlorbenzene do not volatilize rapidly enough to make good the loss incurred by leakage through the clothing. Compounds with lower boiling points such as xylene mixed with fuller's earth might be used successfully as sachets, but the expense of treatment is prohibitive.

LOUSE POWDERS

The most successful louse powders in use at the present time contain naphthalene, and the most favored on the Western front seem to be NCI[1] powder made of naphthalene, 96 per cent; creosote, 2 per cent; iodoform, 2 per cent. The chief objections to this powder are:[9] first, that it is moist, and hence difficult to dust through the clothing, and, second, it is inclined to burn the skin particularly in the fork of the legs.

In the studies of Kinlock[9] and in my own work it was found that the combined powder was more toxic to the lice than any one of its constituents. Experiments show that a saturated solution of naphthalene in creosote was more toxic than creosote alone. The same is true of a saturated solution of iodoform in creosote. Creosote saturated with both iodoform and naphthalene is more toxic than creosote alone, but not as toxic as either creosote-naphthalene, or creosote-iodoform. Just as in fractional distillation, the lower fraction of the liquid, carries over with it small quantities of the higher boiling liquid, so in evaporating, the creosote carries over portions of the naphthalene or iodoform. This fact appears to be the underlying principle which governs the toxicity of the NCI powder.

One cubic centimeter of creosote in evaporating will never carry more than one-half gram of naphthalene or one-fourth gram of iodoform, hence there is at least 95 per cent more naphthalene in the NCI mixture than is required. In

the experiments where talc was substituted for a large part of the naphthalene, a dry powder, easy to dust through the clothing was obtained, which powder retained its dryness even when a much larger percentage of creosote was added. Using talc as a basis, a number of new powders were made up containing creosote or a liquid of about the same volatility and a slightly volatile solid.

TABLE II

COMPOSITION OF POWDERS	PER CENT KILLED IN			
	5 MIN.	10 MIN.	20 MIN.	30 MIN.
NCI (naphthalene 96%, creosote 2%, iodoform 2%)			66	100
Talc 20 grams, naphthalene ½ gram, creosote 1 c.c., iodoform ½ gram			66	100
Talc 20 grams, creosote, 1 c.c.			0	100
Talc 20 grams, creosote 1 c.c., naphthalene ½ gram		66	100	100
Talc 20 grams, creosote 1 c.c., iodoform ½ gram		0	100	100
Talc 20 grams, creosote ½ c.c., oil of sassafras ½ c.c.				0
Talc 20 grams, creosote ½ c.c., amyl alcohol, ½ c.c.			0	100
Talc 20 grams, creosote ½ c.c., methyl salicylate ½ c.c.			0	100
Talc 20 grams, creosote ½ c.c., carbolineum ½ c.c.			0	0
Talc 20 grams, creosote ½ c.c., crude phenol ½ c.c.	66	100	100	100
Talc 20 grams, creosote 1 c.c., chloretone ½ gram		33	100	
Talc 20 grams, methyl salicylate 1 c.c., chloretone ½ gram			0	
Talc 20 grams, creosote 1 c.c., alpha-naphtylamine ½ gram			66	
Talc 20 grams, methyl salicylate 1 c.c., alpha-naphtylamine ½ gram			33	
Talc 20 grams, creosote 1 c.c., paranitrophenol ½ gram		33	100	
Talc 20 grams, methyl salicylate 1 c.c., paranitrophenol ½ gram			66	
Talc 20 grams, methyl salicylate 1 c.c., iodoform ½ gram			0	
Talc 20 grams, methyl salicylate 1 c.c., naphthalene ½ gram			0	
Talc 20 grams, methyl salicylate 1 c.c., quinone ½ gram		100	100	
Talc 20 grams, methyl salicylate 1 c.c., paranitrobenzylchloride ½ gram			33	
Talc 20 grams, creosote 1 c.c., ortho- and parachlornitrobenzene, ½ gram		66	100	
Talc 20 grams, creosote 1 c.c., picric acid ½ gram			0	
Talc 20 grams, creosote 1 c.c., alpha-naphthol ½ gram		66	100	
Talc 20 grams, creosote 1 c.c., beta-naphthol, ½ gram		66	100	
Talc 20 grams, creosote 1 c.c., paradibrombenzene ½ gram	66	100	100	
Talc 20 grams, creosote 1 c.c., menthol ½ gram			66	
Talc 20 grams, creosote 1 c.c., monochloracetic acid ½ gram	100	100	100	
Talc 20 grams, creosote 1 c.c., chloranil ½ gram			0	
Talc 20 grams, creosote 1 c.c., sulphur ½ gram	100	100	100	
Talc 20 grams, creosote 1 c.c., cumarin ½ gram		66	100	
Talc 20 grams, creosote 1 c.c., camphor ½ gram	0	100	100	
Talc 20 grams, creosote 1 c.c., isoborneol ½ gram	66	100	100	
Talc 20 grams, creosote 1 c.c., monobromated camphor ½ gram	66	100	100	
Talc 20 grams, crude phenol 1 c.c., naphthalene ½ gram		0		
Talc 20 grams, crude phenol ½ c.c., creosote ½ c.c., naphthalene ½ gram		66		
Talc 20 grams, creosote 1 c.c., naphthalene and sulphur to saturation	33	100		
Talc 20 grams, creosote 1 c.c., sulphur to saturation	66	100		

A small piece of cloth bearing the lice was placed on a small glass plate and then covered over with a piece of underwear dusted with the powder to be tested. Comparative tests between lice treated on the arm and on the glass plate showed no difference in the period of time required to kill them. Results of these experiments are tabulated in Table II.

Liquids less volatile or more volatile than creosote are not as successful. Methyl salicylate and crude phenol are probably the best substitutes, but not quite as good. No advantage was noted in the use of a compound which readily sublimes such as iodoform, chloranil, and quinone. Considered from the standpoint of irritation to the skin, ease of dusting through the clothing, effectiveness, and expense, the powder recommended consists of creosote 1 c.c., sulphur ½ gram, and talc 20 grams. This powder could be made up for from 5 to 10 cents a pound and will kill 100 per cent of the lice in 5 minutes. A few lice may show signs of life at the end of five minutes, but will later die even though removed from the powder.

Two points may be mentioned concerning the use of powders to destroy the lice. The soldier in general objects to insect powders, since effective powders are inclined to produce irritation, particularly when the soldier is perspired, while powders which do not burn are of no value. The second point is the enormous quantity of powder which would be necessary to effectively treat the great numbers of men at the front. Using two ounces of powder every day or two, which quantity would be necessary for a thorough job, would mean 1250 pounds to each division of ten thousand men. The use of a powder can only be considered as a supplementary control measure.

IMPREGNATION OF UNDERWEAR

Impregnation of the underwear with active volatile organic compounds would, if the amount of the chemical were large, result in considerable irritation of the skin, greater in fact, than that accompanying the use of louse powders, while smaller quantities would soon evaporate and be ineffective. It might be noted that a number of essential oils have been used with greater or less success.[10, 11, 12, 13] The more volatile essential oils are liable to cause considerable burning of the skin while the less volatile essential oils have but slight advantage over the fixed vegetable oils. Slightly volatile mineral, vegetable and animal oils were studied. The lice are inclined to leave cloth impregnated with these oils, but if forced to remain on them they will die.

Olive oil killed 50% in 4 days.
Cedar oil killed 100% in 20 hours.
Castor oil killed 100% in 92 hours.
Cod-liver oil killed 100% in 68 hours.
Paraffin oil killed 100% in 68 hours.
Liquid petrolatum killed 100% in 20 hours.
Rape-seed oil killed 100% in 44 hours.
Neat's-foot oil killed 50% in six days.
Lard oil killed 100% in 116 hours.
Cotton seed oil killed 50% in 4 days.
Commercial oleic acid killed 100% in 20 hours.
Raw linseed oil killed 100% in 63 hours.
Boiled linseed oil killed 100% in 63 hours.
Whale oil killed 100% in 63 hours.
Fish oil killed 100% in 15 hours.
Peanut oil killed 100% in 39 hours.

From an insecticide standpoint the results are not very favorable although all the oils were used without dilution. Oils might be used as repellants but

underwear thus treated is oily or greasy and loses its absorption properties. The value, therefore, of impregnating the underwear with slightly volatile oils is questionable.

An arrangement by which the soldier would wear outside of his underwear a cheesecloth pajama suit impregnated with a volatile organic compound might be feasible. For this purpose a liquid[14] has the advantage over a solid which would require a solvent in its application and would make the cloth stiff and harsh. Cheesecloth dipped in a saturated solution of sulphur in creosote and wrung out nearly dry is successful. The vapor from the impregnated cheesecloth penetrates through the underwear and kills the lice in from twenty to thirty minutes. Suits could be made here and dipped near the front. Comparatively little of the solution would be used if the suits, after dipping, were passed through a strong wringer. Such suits could be redipped and would be as valuable as a louse powder without as much waste of material and without the danger of skin irritation.

FUMIGATION

Heat has been used to a large extent in destroying lice and eggs in the clothing, while the soldier is taking a bath.[15, 16, 17] Dry heat must be carefully watched and regulated to prevent scorching of the clothing.

140° C. of dry heat kills lice and eggs in about 30 minutes.

160° C. of dry heat kills lice and eggs in about 10 to 15 minutes.

In practice it is difficult to reach so high a temperature and maintain it with a large quantity of clothing. The time available for the work is usually stated as about 15 to 20 minutes. Steam leaves the garments wet at the close of the treatment and is apt to ruin leather. Carbon bisulphide, ammonia, sulphur dioxide[18, 19, 20] and hydrocyanic acid are not able to penetrate all parts of the clothing in the short period allowed, while formaldehyde is but slightly toxic to insects. As previously stated, the volatile organic compounds with the lower boiling points are the least toxic.[7, 8] One chemical, however, is a notable exception. Chlorpicrin or nitrochloroform ($CCl_3.NO_2$) although quite volatile, is very toxic. It also has great penetration. Experiments conducted with this chemical have shown that clothing can be piled into a galvanized iron ash tin, sprinkling chlorpicrin through them at the rate of 4 c.c. to the cubic foot and in fifteen minutes all lice will be dead, while a half hour's fumigation is sufficient to kill eggs in all stages of development. In order to kill the eggs by fumigation in thirty minutes, a small amount of heat is necessary to hasten the evaporation of the chlorpicrin. To each cubic foot of space one liter flask filled with water heated to 80° C. was used in the experiments. A twenty-minute fumigation will destroy a large number of the eggs, particularly the youngest and oldest.

In actual practice the outer garments of the men can be fumigated while they are bathing. Ash tins, or perhaps better, a collapsible metal box such as is used on the Italian front for fumigation[21, 22] could be used as a container. Heated stones placed in the fumigation box or even a heated fumigation chamber might be used. By increasing the heat, the period necessary to kill the eggs could be reduced to fifteen minutes. One to two uniforms may be packed in

each cubic foot and the vapor will still be able to penetrate. Arrangements for opening the box outside would be necessary due to the irritation of the chemical to the eyes, nose, and throat. The operator could wear a gas mask when opening the fumigation box and airing the clothing. Airing for three minutes in the open is sufficient to remove the vapor from the clothing.

By a well organized system of allowing the soldier a bath every week or two, at which time they would receive clean underwear and shirts, while their outer garments were fumigated, together with the use of impregnated suits, or a good louse powder as a supplementary measure to prevent reinfestation, it should be possible to rid the men of lice. The destruction of the lice and eggs in the dirty garments could be accomplished by washing and soaking in a water containing a good insecticide.[23, 24, 25]

In conclusion it might be noted that the object of this paper is to give certain facts which have been ascertained concerning the toxicity of various chemicals to lice. Although several methods of destroying lice have been pointed out, they should not be considered as the solution of the louse problem. The real solution of the problem will rest in the practical application of such principles as are determined in the laboratory, and the success of the application will depend upon the thoroughness of the organization developed for carrying out the work.

SUMMARY

Sachets are not successful.

Talc 20 grams, creosote 1 c.c., sulphur ½ gram is six times as effective a louse powder as NCI, causing less irritation to the skin and, being dry, is easier to apply.

Impregnation of the underwear does not appear promising, but a cheesecloth suit impregnated with saturated solution of sulphur in creosote could be successfully worn outside the underwear.

Chlorpicrin can be used as a fumigant, penetrating the clothing and killing the lice in all parts of the clothing in fifteen minutes and the eggs is thirty minutes. By increasing the heat in the fumigation chamber, the time required to kill the eggs could be reduced.

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THE ETIOLOGY OF SCARLET FEVER*

I. A Study of Organisms Found in the Blood of Scarlet Fever Patients

By R. W. Pryer, D.P.H., and J. B. Kelly, M.S., Detroit, Mich.

THE city of Detroit, as well as many other cities of the central portion of the United States, had an unusually high number of scarlet fever cases during the latter part of the winter and early spring of this year.

At one time there were 1385 cases under quarantine in Detroit. The population of Detroit, according to the United States census method of estimation, is 571,784. However, there is no doubt that the true population of the city is in the neighborhood of 750,000. Even this figure shows a tremendously high case rate per thousand of population.

No one organism has been accepted as the cause of scarlet fever, although a vast amount of work has been done on this disease and many suppositions advanced as to the infective agent.

Chief among these is the theory that the causative organism is a streptococcus. This theory is based largely on the fact that members of this family are usually found present in the throat of scarlet fever patients. However, it has not been definitely proved that streptococci are the cause of the disease.

Other workers have claimed that a protozoon is the infective agent. Still others have thought that the organism causing the disease is a virus of the filterable type, but again definite proof is lacking.

An English worker[7] has carried out an extended piece of work which leads him to conclude that a diplococcus is the infecting organism in scarlet fever; but we are of the opinion that he has not entirely differentiated between what he calls the diplococcus scarlatinæ and the pneumococcus.

More recently Mallony and Medlar[8] have reported the results of an investigation on scarlet fever and describe a bacillus as being the infective agent. They give to this organism the name bacillus scarlatinæ.

The first part of this paper deals with only one of several organisms that we have isolated from the blood of scarlet fever patients. We are doubtful about this organism having anything to do with the disease, but are reporting it in detail in order to show the presence of organisms of the diphtheroid group in the blood of scarlet fever patients, of normal individuals, and of persons sick with various diseases.

Since there was an abundance of clinical material for our purpose, we have worked only with the blood of cases which showed typical symptoms of this disease, and the cases reported here were bled during the period in which there was a marked rash.

These cases were diagnosed scarlet fever by the attending physician and

*From the Laboratory of the Detroit Board of Health.

the diagnosis concurred in by Dr. Ostrander, resident physician at the Detroit Municipal Hospital.

METHOD OF BLEEDING

Patients were bled from one of the veins at the bend of the elbow by means of a sterile Luer syringe, 6 to 8 c.c. of blood being withdrawn. The skin over the area of puncture was treated in one of three ways:

1. Scrubbed with strong soap, then washed with 95 per cent alcohol, and finally painted with tincture of iodine.
2. Soaked for five minutes in a 1:1000 mercuric chloride solution.
3. Painted with tincture of iodine alone.

The third method seems to be as satisfactory as any, and is the one recommended by most workers in this field.

METHOD OF MAKING CULTURES

Three methods were used in making the cultures from the blood.

1. One cubic centimeter of blood was added to tubes of sterile broth made up as follows:

2 tubes of nutrient bouillon, plus 10 per cent tryptic extract.
2 tubes of nutrient bouillon, plus 10 per cent tryptic extract, plus 4 per cent glycerin.
2 tubes of nutrient bouillon, plus 10 per cent tryptic extract, plus 1 per cent dextrose.

One of each was covered with ½ inch of paraffin oil, the other maintained under aerobic conditions.

2. The blood was mixed with an equal quantity of tryptic extract and plates made as follows:

One-half c.c., 2 c.c., and 5 c.c. of mixture plated in duplicate, using agar which was neutral to phenolphthalein. Half of plates were incubated aerobicly; the other half, anaerobicly in an atmosphere of hydrogen.

3. The blood was divided into two portions: One portion added to tryptic extract as before, the other portion being mixed with the minimum amount of sodium oxalate solution which prevents clotting. One-half c.c. and 2 c.c. plates were made in duplicate from each mixture.

These cultures were all incubated at 37° C. for a period of two to three weeks. Tubes showing signs of growth were transplanted on to blood agar slants and on to Loeffler's media. Plates were examined every forty-eight hours and were not opened unless we desired to examine a particular colony, in which case transplants were made as above.

NUMBER OF CASES IN WHICH DIPHTHEROID WAS FOUND

The first nine cases studied were all scarlet fever, and we found that it was possible to isolate a diphtheroid organism from the blood in a majority of the cases. It was soon found, however, that the same thing held true for other diseases and for normal individuals. In all, twenty-four cases of scarlet fever have been studied. These were all in the acute stage of the disease. In nineteen, or 79 per cent, of these cases this diphtheroid organism has been isolated in pure culture.

In six normal individuals a diphtheroid organism, morphologically and culturally identical with the one isolated from the nineteen cases of scarlet fever, has been isolated from the blood of four.

In cases of disease other than scarlet fever this organism was found as follows:

Measles, one case, diphtheroid isolated.

Diphtheria, five cases, diphtheroid isolated in two cases.

Pneumonia, four cases, not found.

Typhoid fever, one case, not found.

MORPHOLOGY OF ORGANISM

This diphtheroid is a very pleomorphic organism, varying from coccus forms to a fairly long bacillus. Our experience with this organism has been similar to

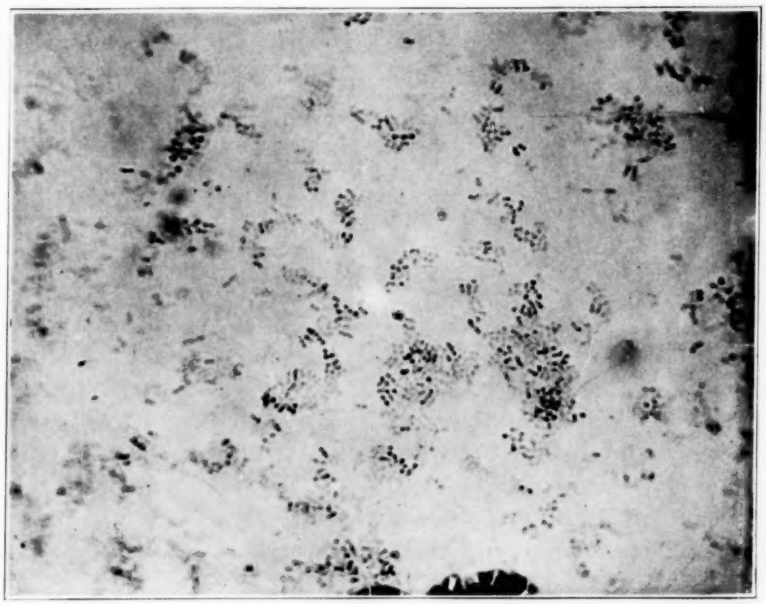

Fig. 1.

other workers with diphtheroids of this class, in that our first cultures were plated many times in order to separate the cocci from the bacilli, and all the time we were working with a pure culture.

In older cultures coccus forms predominate, while in young (18- to 24-hour) cultures the bacillus forms predominate.

The bacilli are usually of the barred type and tend to be shorter and plumper than B. diphtheriæ. This is particularly true if they are grown in media containing serum. However, bipolar staining forms, very similar to B. diphtheria, are frequently seen; and these forms are observed more frequently in young cultures on nutrient agar.

The organism stains readily with the ordinary stains. It is strongly gram-positive and is slightly acid-fast. Loeffler's methylene blue is the best stain. No spores can be demonstrated by any of the staining methods; no motility was observed.

Fig. 1 shows a preparation made from a culture eight days old. It will be noted that the coccus forms predominate.

This organism is nonpathogenic for mice, rats, guinea pigs, and rabbits. Its pathogenicity for monkeys has not been tested.

No agglutination has been observed either with serum of scarlet fever patients or normal serum.

Ten minutes at 70° C. kills this organism, provided an even emulsion is used in making the test.

CULTURAL CHARACTERISTICS

This organism grows readily on solid media at 37° C., after the first culture is obtained. Frequently the plates made with the blood direct require nearly three weeks before the colonies of the diphtheroid can be found. After the first transplant is made, the growth at eighteen hours is heavy.

Growth at room temperature is very slow and scanty.

Growth on gelatin is very slow, media not liquefied.

The growth in liquid media is slow, but heavy after several days' incubation.

No gas is formed in any sugar media. Acidity increases slightly in the following sugar media: dextrose, levulose, sucrose, lactose, maltose, dextrine and mannite.

The organism is a facultative anaerobe.

CONCLUSION ON DIPHTHEROID

There is no question that the diphtheroid group of organisms contains many different varieties. Mellon,[1] in a recent article, makes the statement that he believes there are forty-five different varieties of diphtheroids.

Fox[2] has pictured many different forms of these organisms.

Bunting and Yates[3] have reported an organism of this group as being the etiologic factor in Hodgkin's disease and call it *corynebacterium hodgkini*. The description of the organism reported by these men corresponds very closely with the organism reported in this paper.

A LARGE POLYMORPHIC ORGANISM ISOLATED FROM THE BLOOD OF A MAN DYING OF SCARLET FEVER

Blood cultures were made of one case of scarlet fever shortly before death. After about ten days' incubation, one colony of rather unusual appearance was found on one plate. Direct smears made from this colony, stained with Loeffler's methylene blue, showed what at first was considered a very large diplococcus. Cultures made from this developed a heavy growth after 24 hours in the incubator, blood agar being used for subcultures in this case. Smears made from these subcultures showed many bacillus-like forms as well as very large diplococci. The growth in these cultures being mucus-like, it was thought at first that we might be dealing with a member of the mucus capsulatus family. This was not the case, as a more thorough study of the cultural and morphologic characteristics of the organism plainly showed.

MORPHOLOGIC AND STAINING CHARACTERISTICS

This organism varies from coccus-like forms to fairly long bacillary forms. Length, 2 to 8 *u*. Breadth, 2 to 4 *u*. It is nonmotile, and apparently does not form spores.

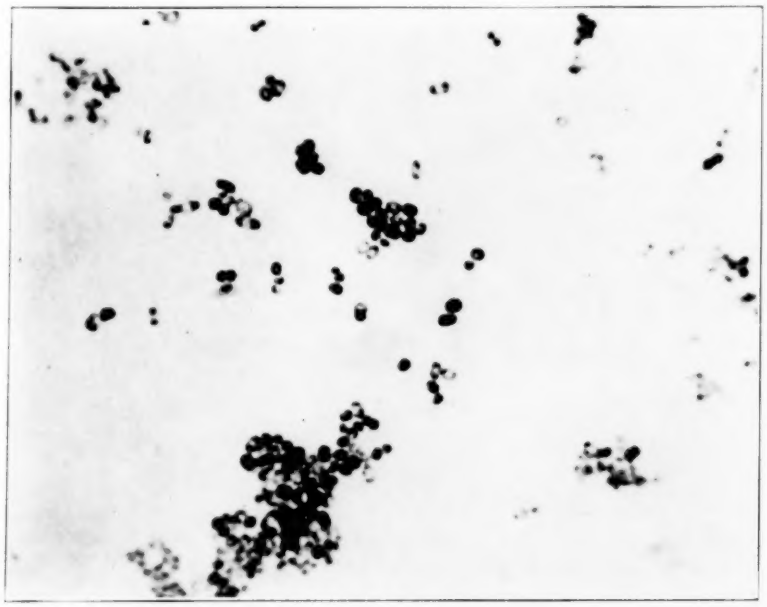

Fig. 2.

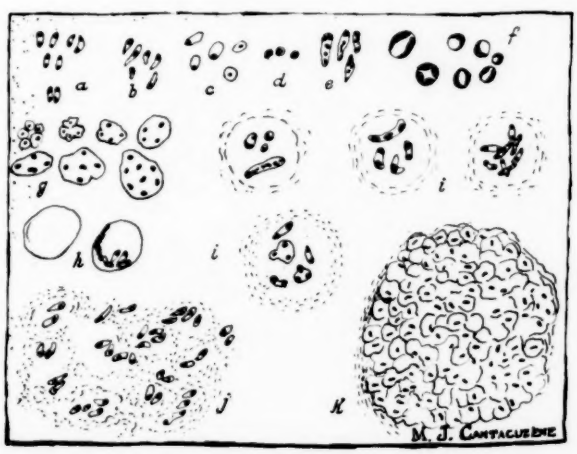

Fig. 3.

It stains readily with the simple stains and at first glance looks like a mixture of very large diplococci and bacilli staining heavily at each end and with a central faintly staining area.

With Giemsa's or Romanovsky's stain, the organism shows a beautiful differentiation, the ends staining bluish purple, a central unstained vacuole, and,

somewhere within this unstained area, a well-defined red spot which looks very much like a nucleus. This is shown in Fig. 2.

Older cultures show longer forms, the red spot lengthening and becoming more irregular in form.

CULTURAL CHARACTERISTICS

This organism grows best at 37° C., growth at room temperature being very much slower. Twenty-four hours at 37° C. on blood agar gives a heavy raised growth, mucus-like in appearance and regularly defined gray colonies. After several days in the incubator, the growth flattens out and is almost invisible, while the culture gives off a pronounced putrid odor.

Gelatin growth is poor; no liquefaction observed after three weeks.

In plain bouillon, growth is practically nil.

In sugar media, dextrose, levulose, maltose, mannite, sucrose, lactose, and dextrine, no gas is formed and after ten days the cultures are much less acid than at first.

PATHOGENICITY FOR ANIMALS

Rats, guinea pigs and rabbits are apparently unaffected by injection of this organism when made either intraperitoneally or subcutaneously. Mice are killed after about four days. Further work on this, as well as on toxin formation, is under way and will soon be reported.

SUMMARY

We realize that we have not proved that this organism is the cause of scarlet fever, but we do think that it is worthy of much further work. Class,[4] several years ago, described a diplococcus that he found in the throats of scarlet fever patients. Mallory[5] has described a protozoon which he found in the skin of scarlet fever patients. Cantacuzene[6] gives a very brief description of an organism found in the throat of a scarlet fever patient and claims to have produced in monkeys a disease very similar to scarlet fever by injection of these cultures.

From the description of Cantacuzene, the organism found by him in the throat is similar to, if not identical with, the one we have described, and which we isolated from the blood in a fatal case of scarlet fever. Fig. 3 is a reproduction of a cut in Cantacuzene's article, and many of these forms can be seen in the microphotographs shown by us in Fig. 2. This work will be continued.

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THE FACTORS OF COAGULATION IN THE BLOOD IN CERTAIN PATHOLOGIC CONDITIONS*

By Dorothy Foster Pettibone, B.A., M.S.

Mayo Foundation, Rochester, Minn.

ELABORATE studies have been made in the past on the various factors which concern the coagulation of blood. Howell[7] has outlined minutely the individual constituents and their respective functions in normal blood. According to Howell, there are five factors in coagulation, that is, prothrombin, fibrinogen, calcium salts, thromboplastin and antithrombin, all of which may show variations in disease. These are all normally present in the circulating blood except thromboplastin, which is contained in all tissue juices, as well as in the formed elements of the blood. On injury of the vessel, therefore, thromboplastin is liberated from the surrounding tissue and the platelets and frees the blood of antithrombin, allowing the prothrombin to act with calcium and the thrombin to unite with fibrinogen and form fibrin. According to Morawitz (quoted by Drinker and Hurwitz),[3] thrombokinase transforms thrombogen, which normally circulates in the blood, to prothrombin. Prothrombin is then changed to thrombin by calcium salts. Thrombin reacts with fibrinogen and produces fibrin. Thrombogen has not been differentiated in any way from prothrombin.

Since the common acceptance of these underlying principles, much work has been done on these same factors in various pathologic conditions with special reference to hemorrhagic disturbances. Methods have been devised whereby these separate factors may be isolated and quantitatively estimated. I have studied a group of cases with special emphasis on prothrombin calcium salts, and platelets. Among these 45 cases, are 2 of hemophilia, 7 of myelogenous leucemias, 2 of purpura, 9 of epilepsy, 12 of jaundice (10 obstructive and 2 hemolytic) and 13 of a miscellaneous group.

HEMOPHILIA

Many workers have reported observations on the blood of hemophiliacs, and the facts they note have agreed for the most part. Howell,[8] in his prothrombin tests, proved that one may by this comparative method readily distinguish between hemophiliac and normal bloods. Sahli believes that the condition is due to deficient thromboplastic material, but there has been suggested as yet no satisfactory method for determining the amount of this substance. The method I have used for determining prothrombin time, is that of Howell.[8] Draw 8 c.c. of blood into a sterile syringe, previously rinsed out with normal salt solution, and express into a tube containing 1 c.c. of a 1 per cent potassium oxalate. Invert the tube once to mix thoroughly and centrifugalize for fifteen minutes. In a series of small tubes place graduated amounts of 0.5 per cent calcium

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chloride, beginning with 2 drops and stopping at 8 drops. Add to each tube 5 drops of the plasma and take time of coagulation (see Table I).

We know by this test that in hemophilias the prothrombin-antithrombin balance is upset, but it is not certainly proved that it is the actual amount of prothrombin that is altered. Addis[1] believes the prothrombin to be present in normal amounts in these cases, but it is so altered as to require longer time for activation to the thrombin present. An excess of antithrombin is present only as a result of decreased prothrombin.

TABLE I

TUBE NO.	1	2	3	4	5	6	7
Calcium chloride	2 drops	3 drops	4 drops	5 drops	6 drops	7 drops	8 drops
Plasma	5 drops	5 drops	5 drops	5 drops	5 drops	5 drops	5 drops
Normal prothrombin time	6 min.	8 min.	8 min.	10 min.	10 min.	12 min.	14 min.

The coagulation time of hemophilia is always long. In estimating this time accurately there are many difficulties to consider. The method used is that described by Lee and White.[5] One cubic centimeter of blood is drawn from the vein into a sterile syringe previously washed with normal salt solution, and expressed into a Wassermann tube which has also been rinsed with normal salt solution. Care must be taken to leave salt solution in the needle before puncture as the admission of air bubbles greatly hastens the coagulation of the blood. The needle is removed so as not to break up the platelets in expressing the blood, and the tube is rotated endwise every thirty seconds till the clot adheres to the tube when inverted. Cohen[2] has shown how necessary are absolute cleanliness, uniformity of apparatus, exact amount of blood, and constant temperature.[11] The time of coagulation greatly depends on the extent of contact to the glass; it may be greatly hastened by any shaking of the tube. The hemophiliac blood may become gelatinous in a comparatively short time, but it is easily noted that the clot is not firm and that its disturbance results in its breaking down. Minot and Lee[13] found that the clots often formed as quickly as normal but never became as firm. If this first clot is removed, a second clot will form; this is also an imperfect clot and may be removed. It has been observed recently by Lee, but not as yet published, that six and eight clots can be removed in succession from hemophiliac blood while that of a normal blood will seldom form a second clot. I was able to remove four clots from the blood in one case, while in normal controls I have never removed more than two.

Case 185593 is worthy of special attention. This patient had a definite history of familial hemophilia; his coagulation time was exceedingly long, but his prothrombin time was within normal limits and his platelet count was 115,000 per c. mm. His mother's blood was then examined and found to have an exceedingly long prothrombin time, though a normal coagulation time. When the prothrombin time was determined, the material was invertible though not as solid and set as in normal sera. Workers in this country have been looking for just such findings, but have not made the actual observations.

Case 182535. In this case there was a coagulation time of 73 minutes, and

after transfusion the coagulation time was reduced to 34 minutes. The prothrombin time was not estimated because of difficulty in procuring blood.

Wright has attributed the hemophiliac condition to a calcium deficiency. Hurwitz and Lucas,[9] in studying five cases in detail, observed the characteristic delay in coagulation and a constant deficiency in prothrombin; other factors of coagulation, however, were present in normal amounts. In the case shown in Table II no appreciable difference in amount or activity of calcium was observed.

TABLE II

HEMOPHILIA

CASE	PROTHROMBIN TIME (CALCIUM CHLORIDE .5%)							COAGULATION TIME	CALCIUM TIME	PLATELET PER C.MM.
	2 drops	*3 drops*	*4 drops*	*5 drops*	*6 drops*	*7 drops*	*8 drops*			
183593	0	0	12 min.	10.5 min.	10.5 min.	10 min.		93 min.	3 hrs. (imperfect clot)	115,000
	24 hours after transfusion:									
	0	0	75 min.	25 min.	38 min.	39 min.		10 min.	12 min.	171,000
Mother of patient	99 min.	99 min.	Lost	—	—	87 min. (imperfect clot throughout)		8 min.	11.5 min.	202,000

EPILEPSY

The results of numerous observations on the blood of patients with epilepsy have been fairly uniform with the exception of the results concerning coagulation time, and these have been most varied. It has been found by several workers to be greatly shortened, while Turner believes the time to be greater in cases of epilepsy. Thorn[14] reports a series of 203 patients with epilepsy whose coagulation time he determined in the method outlined by Lee and White.[10] Ninety-two per cent of these patients fell within normal limits, 5.5 per cent fell under the minimum, and 2.5 per cent were over the maximum. Two and one-half to 14 minutes marked the extremes of the series. Using the same method for determining coagulation time, I studied a series of 9 patients presenting a range of from 4 to 15 minutes, while controls of normal persons ranged from 4 to 12 minutes.

It seems reasonable to conclude that there is no change in the coagulation time of the blood of patients with epilepsy. A great deal depends on the technic, and the differences occurring among normal persons vary in a similar manner.

It is believed that patients with epilepsy may be benefited by calcium lactate. Two patients have returned after a period of from six weeks to two months of the administration of calcium, and were found to have enough calcium in the blood to produce coagulation in less time than by the addition of calcium chloride (Table III).

TABLE III

EPILEPSY

CASE	PROTHROMBIN TIME (CALCIUM CHLORIDE .5%)							COAGULATION TIME	CALCIUM TIME	PLATELET PER C.MM.
	2 drops	*3 drops*	*4 drops*	*5 drops*	*6 drops*	*7 drops*	*8 drops*			
184,006	Not done							11 min.	Not done	Not done
185,070	Not done							7 "	5 min.	Not done
186,346	Not done							6 "	4 "	Not done
186,105	0	26 min.	25 min.	26 min.	27 min.			8 "	14 "	Not done
188,502	0	19 "	15 "	15 "	16 "			12 "	8 "	Not done
189,332	8 min.	8 "	7 "	7 "	7 "			9 "	10 "	Not done
191,596	10 "	10 "	10 "	9 "	9 "	9 min.	9 min.	4 "	4 "	178,000
186,248	Not done							15 "	Not done	Not done
192,424	6 "	7 "	8 "	8 "	9 "	9 "	9 "	6 "	5 min.	215,000

HEMORRHAGIC PURPURA

It has been noted by many that the normal retractability of the blood clot depends on the presence of platelets. Duke[4] proved experimentally that platelets may leave behind them the power to clot but must be present to produce retraction of the clot. I have noted two cases in which the platelet count was 14,000 per c.mm. and no retraction of the clot occurred in eighteen hours. They were typical cases in all respects.

TABLE IV

CASE	PROTHROMBIN TIME (CALCIUM CHLORIDE .5%)							COAGULATION TIME	CALCIUM TIME	PLATELET PER C.MM.
	2 drops	*3 drops*	*4 drops*	*5 drops*	*6 drops*	*7 drops*	*8 drops*			
191,562	5 min.	5 min.	6 min.	6 min.	8 min.	10 min.	10 min.	5 min.	5 min.	14,000
157,449			Not taken					3 "	3 "	14,000
	After transfusion:									139,000

Hurwitz and Lucas[9] are convinced that the chronic purpuras do at times present abnormalities other than platelet deficiency. In two patients under their observation for more than six months, they noted fluctuations in the amount of circulating antithrombin. I have not estimated antithrombin in any of my series.

JAUNDICE

It has long been an established fact that in certain types of jaundice there is a definite delay in coagulation time. Morawitz and Bierich believed that the altered coagulation time was independent of the intensity of the jaundice. Lee and Vincent[11] note that this delayed coagulation does not show up until five or six weeks after the onset of jaundice. The good effects of calcium treatment are felt after several days and the case is deemed safely operable when the calcium time reaches seven minutes. This time is estimated according to Lee and Vincent's[11] technic. One cubic centimeter of the patient's blood is drawn

TABLE V

OBSTRUCTIVE JAUNDICE

CASE	PROTHROMBIN TIME (CALCIUM CHLORIDE .5%)							COAGULATION TIME	CALCIUM TIME	PLATELET PER C.MM.
	2 drops	*3 drops*	*4 drops*	*5 drops*	*6 drops*	*7 drops*	*8 drops*			
160,395 Carcinoma	8 min.	10 min.	10 min.	10 min.	10 min.	13 min.	13 min.	8 min.	5 min.	212,000
183,889 Traumatic	19 "	20 "	20 "	21 "	34 "	15 "		15 "	11 "	200,000
								After calcium treatment 12 min.	20 min.	
189,394 Gallstones	30 "	16 "	15 "	16 "	16 "			4 "	6 "	253,000
190,480 Carcinoma	13 "	11 "	11 "	11 "	9 "	10 "	11 "	10 "	6 "	212,000
125,271 Traumatic	5 "	5 "	5 "	6 "	6 "	7 "	8 "	5 "	5 "	194,000
192,733 Gallstones	6 "	6 "	6 "	9 "	9 "	9 "	9 "	8 "	8 "	262,000
194,975 Traumatic	21 "	19 "	18 "	16 "	18 "	20 "	24 "	21 " (clots)	14 "	209,000
96,625 Carcinoma	0 "	36 "	25 "	18 "	19 "	20 "		20 "	8 "	208,000
190,648 Abscess from ruptured gall bladder	9 "	8 "	7 "	7 "	7 "	9 "	9 "	4 "	6 "	210,000
192,750 Gallstones	10 "	10 "	13 "	15 "	15 "	16 "	18 "	9 "	12 "	228,000

into a sterile syringe previously rinsed with salt solution and expressed, without the needle, into a small tube containing 6 drops of a 0.5 per cent calcium chloride solution. The tube is inverted every thirty seconds and the calcium time is taken at the point of coagulation. The test is the same as for coagulation time with the addition of calcium chloride. It has been found that too much as well as too little calcium will serve to delay the process of coagulation.

Blood platelets were isolated in such cases by Lee and Vincent and were found to act normally both in the formation and retraction of the clot.

It has been found experimentally that bile has an inhibitory effect on the formation of thrombin. Bile will entirely prevent coagulation *in vitro* even in the presence of the optimum amount of calcium. It is probable, however, that bile never becomes so concentrated in the blood of jaundiced patients (Tables V and VI).

TABLE VI

HEMOLYTIC JAUNDICE

CASE	PROTHROMBIN TIME (CALCIUM CHLORIDE .5%)							COAGULATION TIME	CALCIUM TIME	PLATELET PER C.MM.
	2 drops	*3 drops*	*4 drops*	*5 drops*	*6 drops*	*7 drops*	*8 drops*			
192,817	9 min.	9 min.	10 min.	13 min.	14 min.	14 min.	15 min.	14 min.	17 min.	131,000
190,364	5 "	7 "	7 "	7 "	7 "	9 "	9 "	9 "	5 "	200,000

MYELOGENOUS LEUCEMIA

Little note has been taken in this connection of the blood of myelogenous leucemia. In my series of seven patients it will be seen that there is present a tendency to prolonged prothrombin times, although the coagulation times are quite short. Cohen[2] has shown experimentally that a marked leucocytosis very definitely delays coagulation.

It is evident that these patients have the optimum amount of calcium as the addition *in vitro* of 3 drops of calcium noticeably delays coagulation (Table VII).

TABLE VII

MYELOCYTIC LEUCEMIA

CASE	PROTHROMBIN TIME (CALCIUM CHLORIDE .5%)							COAGULATION TIME	CALCIUM TIME	PLATELET PER C.MM.
	2 drops	*3 drops*	*4 drops*	*5 drops*	*6 drops*	*7 drops*	*8 drops*			
192,443	7 min.	7 min.	8 min.	9 min.	9 min.	9 min.	10 min.	8 min.	12 min.	196,000
192,150	16 "	13 "	12 "	12 "	12 "	14 "	14 "	8 "	8 "	194,000
177,183	Not done							12 "	14 "	270,000
176,684	17 min.	13 min.	15 min.	2 hrs.	2 hrs.			8 "	9 "	185,000
186,558	14 "	12 "	13 "	15 min.	15 min.			8 "	11 "	Too many myelocytes
162,899	Not done							18 "	16 "	280,000
180,499	15 min.	15 min.	20 min.	20 min.	20 min.	21 min.	25 min.	7 "	6 "	188,000

MISCELLANEOUS CASES

Drinker and Hurwitz[3] found prothrombin to be slightly diminished in all patients with pernicious anemia. I found that to be true in the one patient examined in the Mayo Clinic. The platelet counts are, however, relatively normal.

Three of the splenomegaly cases presented delayed prothrombin times. Coagulation times were rather long, but the addition of calcium tended to retard.

Among this miscellaneous group there were several types of conditions. As control cases they showed nothing abnormal in their factors in coagulation (Table VIII).

TABLE VIII

MISCELLANEOUS

CASE	PROTHROMBIN TIME (CALCIUM CHLORIDE .5%)							COAGULATION TIME	CALCIUM TIME	PLATELET PER C.MM.
	2 drops	*3 drops*	*4 drops*	*5 drops*	*6 drops*	*7 drops*	*8 drops*			
183,556 Diabetes	30 min.	23 min.	16 min.	17 min.	11 min.	15 min.		4 min.		
76,654 Splenic anemia?	0	40 "	25 "	25 "	23 "			16 "	22 min.	288,000
192,553 Splenic anemia	11 "	13 "	14 "	15 "	15 "	17 "	19 min.	9 "	10 "	177,000
192,503 Cirrhosis of liver? with splenomegaly	7 "	9 "	9 "	12 "	14 "	18 "	18 "	10 "	13 "	
190,774 Localized tuberculous splenomegaly	9 "	8 "	7 "	7 "	7 "	7 "	8 "	7 "	6 "	197,000
192,719 Pernicious anemia	14 "	20 "	22 "	24 "	30 "	30 "	30 "	5 "	4 "	204,000
185,197 Pernicious anemia	Not done							6 "	Not done	157,000
189,232 Lues II	Not done							Not done	Not done	269,000
193,565 Polycythemia	8 min.	8 min.	7 min.	6 min.	6 min.	6 min.	6 min.	8 min.	9 min.	115,000
190,423 Migraine	8 "	8 "	8 "	7 "	7 "	9 "	9 "	7 "	9 "	208,000
191,091 Biliary cirrhosis?	17 "	17 "	16 "	16 "	16 "	16 "	16 "	15 "	10 "	223,000
192,121 Biliary cirrhosis?	5 "	5 "	5 "	5 "	5 "	6 "	6 "	7 "	8 "	255,000
194,103 Hypernephroma	10 "	12 "	14 "	16 "	15 "	15 "	16 "	9 "	8 "	200,000

NOTES ON TECHNIC

In doing these prothrombin tests we have noted that on reducing the amount of blood used to just one-half, the results were identical and the test more economical,—the amount of potassium oxalate being reduced proportionately. Care must be taken to use the same needle for measuring calcium and serum, for the time varies appreciably if the size of the drops is not uniform. I have also done parallel prothrombin tests on several cases, using .5 c.c. of potassium oxalate to 8 c.c. of blood. In the cases of jaundice great difficulty was experienced in avoiding a clot while centrifugalizing the blood. If a clot had formed, the serum it expressed would not clot on the addition of calcium. In the cases in which the serum did not clot while spinning, however, a shorter prothrombin time was manifested when only .5 c.c. of oxalate was used. In these cases there was not sufficient oxalate to precipitate all the calcium, and in one case there was so much native calcium that the optimum amount of calcium brought the shortest time of clotting in the first tube followed by an excess of calcium in the rest of the tubes and a consequent retarding of the clotting-process.

The method employed for platelet enumeration is that described by Wright and Kinnicutt.[16] Blood was mixed with the diluting fluid in the proportion of one to a hundred by means of the ordinary red blood corpuscle pipette and counted in the blood counting chamber with a high dry objective. The diluting fluid consisted of two parts of an aqueous solution of brilliant cresyl blue (1:300) and three parts of an aqueous solution of potassium cyanide (1:1400).

These two solutions must be kept separately and mixed and filtered only as used or a precipitate forms and obscures the platelets. In order to get an even distribution of platelets, it is well to fill the chamber at once after mixing blood and fluid in the pipette.

I found that waiting an appreciable length of time and shaking as for red blood corpuscles and white blood corpuscles is not satisfactory. Wright[17] demonstrated several years ago that platelets tend to adhere to any foreign body and form clumps. This perhaps explains the reduced counts after shaking the pipette. Shaking also tends to form larger and denser clumps which makes the count very inaccurate. There are three main requisites for reliable platelet counts; namely, the red corpuscles must be laked, the protoplasm of the leucocytes well stained, and the platelets evenly distributed.[4]

After the counting chamber has been filled, it may stand for hours before making the count. It must surely stand ten minutes to allow the platelets thoroughly to settle. The cresyl blue solution will keep indefinitely on ice, which prevents growth of yeasts. Potassium cyanide should be made up fresh at least every ten days. It must be made of pure potassium cyanide not undergoing degeneration.

Duke found platelet counts fairly constant in normal persons but fluctuating to extremes in pathologic conditions. By various experimental devices he showed that the platelet count fluctuated in exact proportion to the degree of toxicity. He found that the injection of diphtheria toxin in small doses raised the count and that the same toxin in lethal or nearly lethal doses decreased the count rapidly. He concluded from his experiments that when increased counts were associated with pathologic conditions the case was usually mild. In other words, the toxins act as irritants or poisons according to the size of dosage. Duke[6] bore out his deductions in man; his average counts of patients in febrile condition was 114,000 per c.mm.—the convalescent counts going as high as 750,000 per c.mm.

SUMMARY

1. The technic of the various laboratory methods used is described.

2. There was a marked calcium deficiency in jaundice cases of several weeks' duration. Platelets were present in normal numbers.

3. The blood of hemorrhagic purpuras was deficient in platelets. There was no retraction of the clot. Estimation of platelets was of value in differentiating between hemorrhagic purpuras and hemophilias.

4. The coagulation time of epileptic blood was within normal limits.

5. Prothrombin was slightly diminished in pernicious anemia.

6. There was a tendency in myelocytic leucemias for prothrombin time to be prolonged beyond the normal limits of from six to fourteen minutes, although the coagulation time was within normal limits.

7. There was a characteristic delay in coagulation in the blood of hemophiliac cases. There was also a deficiency in prothrombin. Platelets were present in normal numbers and have normal retractile powers. There was apparently no deficiency in calcium.

8. One patient of special note among the hemophilias had an extremely

long coagulation time though his prothrombin time was within normal limits. His mother had a normal coagulation time but a markedly delayed prothrombin time.

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CHRONIC TONSIL INFECTIONS*

By Josiah J. Moore, M.S., M.D., Chicago, Ill.

As an introduction to the discussion of the pathology of the tonsils, the following remarks on their anatomy would seem pertinent.

The faucial tonsils are two symmetrically placed prominent bodies situated between the anterior and posterior pillars of the soft palate and covered by a layer of epithelium which is continuous with the mouth cavity. The prominence of the bodies varies at different ages and in individuals of the same age. Maclachlan in his series gives the average size as 2.5x1.8x1.5 cm. In 250 pairs of tonsils examined in a routine manner I found the average size to be somewhat smaller or 2.3x1.5x1.0 cm.

Each tonsil is enclosed in a fibrous capsule which surrounds it completely except on the inner surface where the openings on the mucosal surface are found. The mucous surface covering the tonsil varies considerably in appearance. It is usually convex. Depending upon the character of the mouths of the crypts it may be either ragged or smooth.

The crypts of the tonsil are diverticula of the buccal cavity lined by squamous epithelium. They are ten to fifteen in number extending into the tonsil almost to the capsule where they end blindly. The contents of the crypt are saliva, food debris, cell debris and bacteria, the contents of the mouth cavity proper. Branching from the main crypts are numerous smaller crypts which extend for a considerable distance into the tonsillar substance.

The tonsil substance proper is composed of trabeculæ, stroma, follicles, blood and lymph vessels and the individual cells of the tonsils.

The trabeculæ are fibrous bands arising from the capsule which extend across the tonsil becoming smaller toward the buccal surface. They carry large vessels and lymphatics, and give partial origin to the stroma of the tonsil. The vessels of the trabeculæ are quite large. The stroma is a loose connective tissue structure forming the supporting framework for the tonsil cells.

The follicles make up the greater part of the tonsil substance proper. They consist of numerous collections of cells, similar in structure to the follicles of the lymph nodes. The central cells, constituting about one-third of the follicle, are large with prominent nuclei and abundant cytoplasm. At the periphery the cells are smaller with deeper staining nuclei and less protoplasm. The interfollicular tissue is made up of a very loose reticular stroma containing many fine blood and lymph capillaries and packed with small monouclear cells. Plasma cells are constantly found in the tonsil from the second week after birth.

The blood vessels and lymphatics of the tonsils follow the trabeculæ are similar to those of any other organ.†

*From the Department of Pathology and Bacteriology, University of Illinois, College of Medicine, Chicago, Ill.

†An excellent discussion of the anatomy, physiology and other phases of this subject has been given by Maclachlan: Tonsillitis, University of Pittsburg, School of Medicine, Pub., 1912.

Due both to its position and structure the tonsil is more prone to inflammation than almost any other organ in the body. Owing to the former it comes in direct contact with the various species of bacteria in the mouth cavity. More important, however, is the deposition of these bacteria with food and other debris in the lumina of the crypts. Here sluggish drainage, ideal temperature and moisture conditions, and varying degrees of oxygen pressure furnish the organisms with a splendid opportunity for development. Depending upon the type and virulence of the organism and the resistance of the host, either an acute or a chronic condition results. Although the majority of tonsillar inflammatory conditions arise from irritants reaching the organ from the buccal cavity, some, such as secondary syphilis, are metastatic in nature.

In this discussion on chronic tonsil infections the classification used is based, not upon clinical data, but upon actual changes in the tissue recognized pathologically as chronic inflammation. Various classifications have been suggested, but the following by Semon and Williams[1], based upon the location of the lesion, is at least convenient. (a) Chronic lacunar or cryptic tonsillitis; (b) chronic interstitial tonsillitis; and (c) chronic peritonsillitis. These types correspond with a similar classification of acute conditions to which they are closely related. Either one may precede the other, or they may and frequently do exist simultaneously.

Chronic Lacunar Tonsillitis.—Chronic lacunar tonsillitis is the most common lesion. Changes are found in the lining, size, and contents of the crypts. In chronic lacunar inflammations the epithelium is irregular due to the dilatation of the finer branches of the main crypts, papillomatous masses of keratinized cells, and desquamation of cells. The cavity of the crypts is usually dilated, containing desquamated epithelial cells, cholesterin crystals, polymorphonuclear cells, hyaline material and bacteria. Mycelial growths, or "actinomyces-like bodies," are often found in this condition. The shape of the dilated crypts and the compressed epithelial lining suggests blocking of the mouths.

It is in this type of tonsillitis that the soft, yellowish, foul-smelling, cheese-like particles may be frequently squeezed from the crypts. Not infrequently calcium salts are deposited in such masses, forming tonsilloliths. Occasionally there is a plugging of one of the crypts with an increase in the keratinized epithelial cells, forming an epithelial cyst.

Chronic Interstitial Tonsillitis.—Chronic interstitial tonsilitis is characterized by an increase in connective tissue. Since this is derived from the capsule and trabeculæ, there may be an increase in the size of the trabeculæ or an increase in the amount of stroma in the parenchyma of the organ. These two forms are usually associated. In small hidden atrophic tonsils fibrosis is to be expected, but it is often found in hypertrophied tonsils. It is generally conceded, however, that the hypertrophic finally passes into the atrophic state. Islands of cartilage or bone occasionally appear in the stroma in fibrosis of the tonsils. Here also we see the thick-walled vessels which so often cause hemorrhage in tonsillectomy in this type of inflammation. Chronic lacunar and interstitial tonsillitis are often found together. In 250 pairs of tonsils examined they were found associated in 42 per cent.

Closely associated with these types of tonsillitis is the condition termed hypertrophy of the tonsil. The etiologic factors are numerous. Heredity is thought by some to play an important role. Constitutional or predisposing causes are tuberculosis, syphilis, gout and alcoholism in parents. Local causes, such as recurrent attacks of tonsillitis, are, however, most important. Usually the only distinctive point clinically in the gross appearance of the tonsil is its actual size. Our largest tonsil measured 4x3x1.7 cm.

The most common microscopic picture in the hypertrophy of the tonsil on children is an increase in both size and number of follicles so that they occupy more space than the diffuse lymphoid tissue. The enlargement is in all probability due to hyperplasia of the cells in the center of the follicles. In adults the follicles of the hypertrophied tonsil are usually diffuse and lymphoid tissue with stroma make up the major portion of the tonsil. Practically always some form of inflammation may be found if properly sought. Most commonly this is acute or chronic lacunar tonsillitis. Hyperplasia of the lymphoid tissue was observed in 30 per cent of the series examined.

Plasma cells in large numbers are found in all hypertrophied tonsils, and since these cells are usually indicative of chronic infection, their presence suggests this as a possible etiologic feature in the hypertrophy. Plasma cells are found, however, in abundance in all tonsils, normal or pathologic, though more numerous in the latter.

It is sometimes difficult to differentiate between a fibrotic atrophic tonsil the result of chronic inflammation and one undergoing the normal atrophy. Features of diagnostic importance would be, clinically, the age of the individual and the history of repeated attacks of acute tonsillitis, and microscopically the size and contents of the crypts and the presence of lacunar inflammation.

Chronic Peritonsillitis.—Chronic peritonsillitis is characterized by an increase in the fibrous tissue in the capsule and pericapsular tissue with infiltration of characteristic inflammatory cells. Cartilage and bone are occasionally found in the thickened capsule.

Of special interest as chronic infections of the tonsil, are those of tuberculosis and syphilis. It is questionable whether a diagnosis of primary tuberculosis of the tonsil can be made during life. Statistics on the frequency of primary and secondary tuberculosis of the tonsils may be very misleading even if accompanied by data indicating the presence or absence of tuberculosis in other parts of the body. The frequency of tuberculosis when no other foci are demonstrable varies from 1 to 5 per cent. In patients suffering from pulmonary tuberculosis the tonsils are affected frequently. Wood[2] believes that 90 per cent of tuberculous cervical adenitis is secondary to tuberculous infection of the tonsil. It has been demonstrated, however, that the tuberle bacillus can pass through the tonsil and to the lymph glands, with no apparent injury to the tonsil. Ravenel,[3] by feeding tubercle bacilli to hogs, found that the bacilli passed through the intestinal mucosa to the chyle and mesenteric lymph glands without producing microscopic lesions in the intestines.

In my series of 250 pairs of tonsils, tuberculosis was present in 2.5 per cent. Clinical examination failed to disclose involvement of either the lungs or the

cervical lymph glands. The ages ranged from 6 to 25 years of age. The presence of typical tubercles on microscopic sections was considered sufficient for diagnosis. They revealed caseation, epithelial cells, giant cells, and connective tissue changes. Giant cells alone might be due to a variety of causes, as foreign bodies, cholesterin crystals, and syphilis.

Syphilis of the tonsil may be primary, secondary, or tertiary. According to some the tonsil is more generally involved in extragenital primary lesions because its structure gives greater opportunity for the spirochete to lodge. The percentage varies from 6 to 55 per cent. Women are most frequently affected. The lesion occupies the greater part of the mucous surface, with sharply defined margins and considerable induration. They are usually painless. Enlargement of the lymph glands under the angle of the jaw is always observed.

Secondary syphilis shows itself in the tonsil in the form of mucous patches, occasionally by superficial ulceration.

Tertiary lesions are the gummata or the resulting ulcerations. They are rare. Healing of the gummata results in the formation of much scar tissue with marked induration of the tonsil.

Actinomycosis of the tonsil has been occasionally described. Davis[4], from his own work and from a review of the literature, thinks that no well-authenticated case of primary actinomycosis of the tonsil has been reported. The "actinomyces-like bodies" or granules found in the crypts are not true actinomyces, but consist of streptococci, bacilli, and spirilla growing together in a more or less symbiotic relationship. The bacilli belong to the fusiform group and neither they nor the streptococci have any marked pathogenicity for animals. These granules are in about one out of every three or four pairs of tonsils examined. Probably all tonsils at times contain them.

The bacteriologic flora of the tonsil is somewhat similar in both acute and chronic tonsillitis. The surface bacteria are usually those found upon all surfaces of the buccal cavity and come from the throat, mouth, nose, food and inspired air. Cultures from the surface are of little value in determining the type of organism in the crypts. In the crypts, many varieties of microorganisms have been found, but those predominating are streptococci. Davis[5], isolated hemolytic streptococci in the majority of his series of 133 tonsils, with pneumococcus, staphylococcus, B. diptheriæ, B. influenzæ and B. mucosus capsulatus in a small number of cases. These streptococci are capable of producing arthritis in rabbits. They were isolated from individuals suffering from chronic arthritis, endocarditis, and nephritis, but it is not certain that they were causative factors in all cases. It must be recognized that in almost all tonsils, whether pathologic or not, hemolytic streptococci capable of causing arthritis in rabbits may be isolated from the crypts.

Frequent favorable results following removal of infected tonsils suggest that the absorption of bacteria or their products from the crypts has much to do with the causation of certain chronic lesions. The severity and site of these lesions may depend upon the virulence and specificity of the bacteria and the general and local resistance of the host. Rosenow[6], suggests that certain organisms have an elective affinity for different tissues. Strains of streptococci ob-

tained from appendicitis, ulcer of the stomach, cholecystitis, rheumatic fever, erythema nodosum, myocarditis, endocarditis, and herpes zoster when injected intravenously into animals, rabbits and dogs, elect to produce the specific lesion from which they were isolated in a very high percentage of cases. Not only do strains isolated from the specific lesions, but also strains isolated from the apparent focus of infection, which frequently was the tonsil, give more or less comparable results. He likens a pocket in the tonsils filled with pus, necrotic material, and bacteria to a culture tube having a permeable wall affording abundant opportunity for the entrance of bacteria and their products. The importance of such foci of infection to systemic disease is especially emphasized by Billings[7] in his extensive study.

In a series of rabbits studied, using an organism isolated from a patient with subacute arthritis, endocarditis occurred twice, nephritis three times, and arthritis in all. In a series of forty-five rabbits injected with a hemolytic streptococcus isolated from the tonsillar crypts during an attack of accute tonsillitis in a patient having chronic arthritis, arthritis developed in all the rabbits, endocarditis in 50 per cent, kidney lesions in 15 per cent, intestinal hemorrhage 6.6 per cent, hemorrhage in the stomach in 5 per cent, appendicitis and gall bladder lesions 2.5 per cent, and myocarditis in nearly all.[8]

It is interesting to compare this last group with a group recently injected with a hemolytic streptococcus from a case of acute tonsillitis with no complications. Fourteen rabbits were injected. The percentage of lesions was as follows: arthritis 90 per cent, endocarditis 50 per cent, nephritis 28 per cent, myositis 42 per cent, myocarditis 21 per cent, adenitis 14 per cent and hemorrhages into the gall bladder 17 per cent. The variable number of lesions produced by these different strains possibly illustrates Rosenow's hypothesis that "the tendency to locate electively within a limited range, monotropism, is most highly developed in the nonvirulent strains isolated from chronic lesions." In the more virulent strains from acute lesions this tendency is less highly developed, the lesions occurring over a wider range, "polytropism."

Reference has already been made to the organisms composing the so-called "actinomyces-like bodies" found in the crypts. Pilot and Davis[9], have made anaerobic cultures of many granules found in tonsils and frequently obtained hemolytic and nonhemolytic streptococci of low virulence capable in large doses of producing arthritis in rabbits. On early transplants they grow better under anaerobic conditions than under aerobic conditions. Later they lose this characteristic.

The tubercle bacillus is rarely found in the tonsillar crypts. Occasionally diphtheria and influenza bacilli[10] occur here, where they may persist for a long time. Diphtheroids are not uncommon in the crypts.

The persistence of virulent diphtheria bacilli in the crypts of the tonsils brings up the very important problem of the role played by these organs in disease "carriers." Much has been written on this subject and reference to some of the more recent literature will demonstrate its importance. Friedberg[11] found that persistent diphtheria carriers became negative after removal of the tonsils. In microscopic sections of these tonsils[12] the diphtheria bacilli were seen in

superficial ulcers located usually in the crypts. In nineteen diphtheria carriers Ruh, Miller and Perkins[13] isolated pure cultures of B. diphtheriæ from the tonsillar crypts both before and after tonsillectomy. All these writers recommend the removal of the tonsils in individuals where diphtheria bacilli persist for any considerable period of time.

It is of interest that the crypts of the tonsils may become chronically infected with meningococci. Although in meningococci carriers the organisms are usually isolated from the nasopharynx, Mathers[14] recently has obtained them in several cases from the crypts of extirpated tonsils. Whether removal of the tonsils will free the carriers of meningococci is still a matter of conjecture.

A third disease where the virus may be harbored in the tonsil is poliomyelitis. Rosenow[15] and his coworkers and Nuzum[16] have obtained organisms from the crypts of tonsils in patients with or recovering from poliomyelitis which resemble morphologically and culturally, and produce the same type of paralysis in animals, as the cocci which they isolate from the spinal cord of fatal cases. Sydell[17] concludes that the tonsils and adenoids may be the portals of infection for poliomyelitis. Their experiments suggest that the tonsils may act as the resting place for the virus. Flexner[18] states more conclusively that "the virus of the poliomyelitis has been traced to the respiratory mucous membrane of healthy persons who may act as carriers and has been found in several instances in the tonsils removed by operation several months after recovery from the acute disease, so that the existence of so-called chronic carriers has also been indicated."

In a general way these examples suggest that the infectious organism or virus of many diseases which have as their portals of entry the upper respiratory system, may lodge either permanently, or for a long period in the crypts of the tonsils, thus producing the state which we term a "chronic carrier." The removal of the infected tonsil may aid materially in controlling such diseases.

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LABORATORY METHODS

A RAPID COLORIMETRIC METHOD FOR ESTIMATING GLUCOSE IN URINE*

By Victor I. Isaacson, B.S., New York City

SEVERAL excellent methods are at present in use for quantitatively determining glucose in urine. The procedure described below is reported, however, because it is simple as well as accurate and requires little skill and time. The new method, like many of the older ones, depends upon the fact that alkaline copper sulphate solutions are reduced by glucose at higher temperatures. The quantity of copper sulphate reduced is computed indirectly by determining the amount left unreduced. This latter portion is measured in a colorimeter† by comparing the intensity of its blue ammonia compound with a standard solution of the same substance.

Solutions.—The solutions used are: (1) Concentrated ammonia water. (2) A solution resembling Benedict's qualitative sugar reagent, containing 34.65 gm. copper sulphate with five molecules of water, 100 gm. anhydrous sodium carbonate and 100 gm. sodium citrate in one liter of water. The copper sulphate should be dissolved separately in about 200 c.c. of water and then added to the solution of the other two reagents with stirring. The whole is made up to one liter. Ten c.c. of this solution is equivalent to 50 mg. of glucose.

Standards.—Standard A is made by measuring 10 c.c. of the solution just described into a 25 c.c. volumetric flask, adding 3 c.c. of concentrated ammonia water, diluting to the mark, and shaking until uniformity of intensity is insured. The numbers appearing to the left of the calibrations on the graduated tube (Fig. 2) give the result directly in per cent when working with this standard.

Standard B is half as strong and is prepared by measuring 5 c.c. of the reagent into a 25 c.c. flask, adding 3 c.c. of concentrated ammonia, diluting to the mark, and shaking. When using this standard for comparison, the numbers in the right-hand column on the calibrated tube yield the answer directly in per cent.

Standard C is intermediate in intensity between A and B and is made by mixing equal portions of these two standards. The results for this standard do not appear on the calibrated tube, but may be secured by dividing the sum of the two numbers at any given point on the tube by two. In every case these figures give the quantity of glucose in the specimen *directly in per cent* provided only *1 c.c.* of the specimen was used for analysis. For other quantities the figures read off must be divided by the number of cubic centimeters of urine employed. The standard color solutions are transferred to the ungraduated

*From the Chemical Laboratory of Montefiore Home and Hospital, New York City.

†The apparatus may be obtained from Eimer & Amend, New York City.

tubes, labeled A, B, and C, stoppered tightly, and kept preferably in a cool place.

Technic.—Ten c.c. of the copper sulphate solution is measured into a 7x⅞ inch test tube. Between 10 and 30 mg. of glucose is the most favorable quantity with which to work. If the qualitative test has indicated 1 per cent or more of glucose, 1 c.c. of the specimen is added. For percentages less than one, it is advisable to use 3 c.c. of urine; and for percentages approaching 3.5, it is necessary to reduce the quantity used to ½ c.c., to make the precipitation quantitative. Two glass beads are added to prevent bumping, the test tube is shaken and the contents *boiled* for three minutes over the free flame. About one-tenth of a gram of animal charcoal is added, and the solution is filtered through a folded filter paper into the graduated tube, allowing the first 10 to 15 drops to flow back into the test tube. When all is filtered, the paper is washed with a few drops of water repeatedly until the filtrate is colorless. Three c.c. of concentrated ammonia

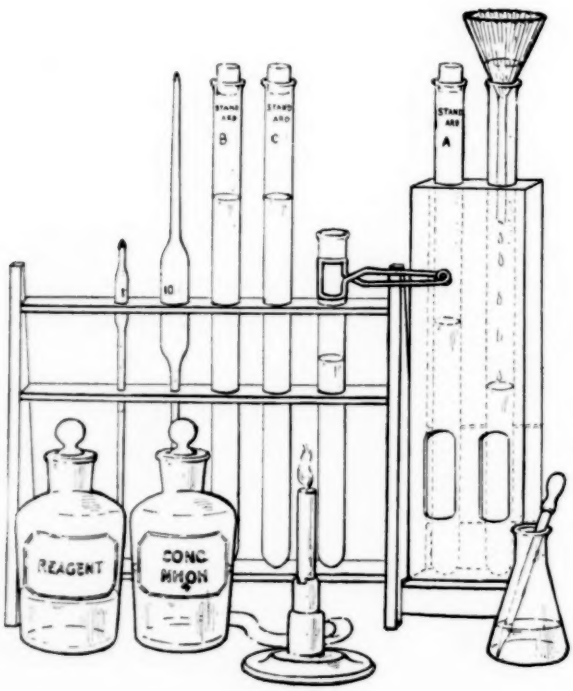

Fig. 1.

Fig. 1.—Sketch of colorimeter, color tubes, and apparatus required in the procedure.

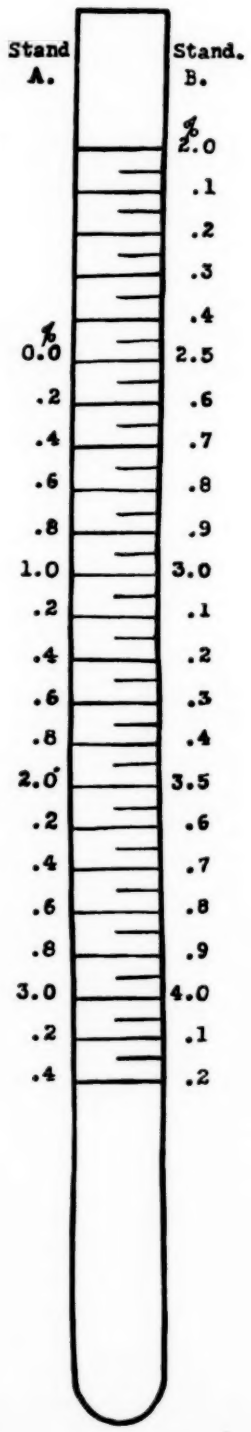

Fig. 2.

Fig. 2.—Comparison tube. Numbers in the left-hand column are read when working with darkest standard. Those in right-hand column for the lightest. The average of two figures on same line gives answer for intermediate standard.

water are added to the contents of the tube to develop the color; the tube is inverted to insure mixing, and water is added from a medicine dropper until the intensity is the same as that of one of the three standards. The reading taken will depend on the standard used.

Although there are no steps that offer any difficulty, a few suggestions are necessary. The amount of citrate in solution influences the intensity of the blue color; the same quantity within one gram should, therefore, be weighed out each time more of the reagent is prepared. One should make no attempt to reduce the time of boiling else the precipitation may not be complete. Care should be taken to refilter the first portion that comes through, since the cloudiness is due to cuprous hydroxide which dissolves in ammonia and yields on oxidation a blue color identical with that formed by cupric salts and ammonia. No trouble is encountered in most cases. When the turbidity persists, several times the quantity of animal charcoal ordinarily used should be added. One should avoid the use of too much wash water since this may make the filtrate of concentrated sugar solutions too light to read. The ammonia water need not be measured accurately. Foaming may be prevented by adding two or three drops of caprylic alcohol.*

The operations may be concisely summarized as follows:

1. Pipette 10 c.c. of the reagent into a test tube.
2. Add ½, 1, or 3 c.c. of urine, depending on concentration.
3. Drop in 2 glass beads, shake, and *boil for three minutes.*
4. Add about $\frac{1}{10}$ gram of animal charcoal and filter, refiltering the first small portion.
5. Add about 3 c.c. of concentrated ammonia water to the filtrate, invert several times and compare.
6. Read the proper figure, and if a quantity other than 1 c.c. of urine was used, divide the figure by the number of cubic centimeters used.

A few time-saving devices have been worked out for use when *several* analyses have to be done at the same time. A beaker of 750 c.c. capacity half filled with water may be used as a heating agent. When the reagent and specimens have been measured, the test tubes are inserted and allowed to stand in the beaker for four to five minutes *from the time the water has begun to boil vigorously.* Bumping can be prevented by introducing into the bottom of the beaker a piece of wire gauze of half inch mesh turned down at the sides. The remaining steps are the same as described above. Filtrations and washings can be performed simultaneously.

A method similar to the one just described was proposed by Autenrieth and Tesdorpf† in 1910 and later modified by Eschle.‡ They proposed the colorimetric measurement of the unreduced portion of a Bang's copper sulfate solution. The quantities used, however, were large (60 c.c.), the reagents costly, and moreover unstable, autoreduction taking place on boiling the solution. Considerable time had to be expended in standardizing the apparatus. Ammonia could not be used to develop the blue color and the faint green color of the copper sulphate

*Specimens which contain albumin must first be heated and filtered, since the protein modifies the blue color to such an extent as to make comparison difficult.

†Autenrieth, W., and Tesdorpf, Th.: München. med. Wchnschr., 1910, lvii, 1780.

‡Eschle, O.: Fortschr. d. Med., 1912, ii, 326.

was not well adapted for comparisons. These difficulties are overcome in the new procedure and a further decided advantage is the use of the same reagent as a qualitative and quantitative solution.

The results of comparative analyses made with the new method and by the Pavy-Kumagawa procedure are recorded in Table I. Analyses of pure glucose solutions further indicate that the new procedure is accurate. A 1 per cent solution gave 1.04 per cent; a 2 per cent solution, 2.04 per cent, 2.08 per cent; and a 3 per cent solution, 3.07 per cent, 3.05 per cent, 3.11 per cent. Above 3 per cent the precipitation is not quantitative and, therefore, it is essential to employ only ½ c.c. of the specimen for such concentrations.

The same solution may be employed as a qualitative reagent. Either 5 c.c. of the reagent and 10 drops of urine, or 10 c.c. of the reagent and 1 c.c. of the specimen should be used to make the test. The mixture should be boiled two minutes over the free flame or left in a beaker of boiling water for five minutes. The use of 10 c.c. of the reagent and 1 c.c. of the specimen, both accurately measured, is advisable if a quantitative procedure is to follow, since measurements need not be repeated. The reagent gives an easily discernible turbidity with a 0.05 per cent pure glucose solution. The results of comparative qualitative tests made with this and with Benedict's qualitative sugar reagent are summarized below. It is evident that its sensitiveness as a qualitative solution enhances the value of the quantitative procedure.

A. Ten c.c. new reagent and 1 c.c. of 0.10 per cent pure glucose solution, marked cloudiness throughout solution in less than a half minute boiling. Five c.c. Benedict's reagent and 1 c.c. same glucose solution, slight reddish precipitate after three minutes boiling but no general cloudiness. Repeated with 9 drops of glucose solution and 5 c.c. Benedict's, boiled three minutes, slight red precipitate.

B. Ten c.c. new reagent and 1 c.c. 0.05 per cent pure glucose solution, one-half minute after boiling commences, turbidity is evident throughout the solution. Five c.c. Benedict's reagent and 1 c.c. of the same glucose solution, several specks of cuprous oxide float about, but they might be easily overlooked.

The following comparative tests were made on urines of diabetic patients. All were tested by using 1 c.c. of the specimen, 10 c.c. of the new reagent and kept in a steam bath for 5 minutes from the time boiling commenced. The quantities used for the test according to Benedict were those recommended by that author.

(a) G. Solution remains clear	Benedict negative.
(b) B. Solution remains clear	Benedict negative.
(c) P. Slight yellow precipitate on standing	Benedict negative.
(d) M. Very slight yellow precipitate settles out on standing	Benedict negative.
(e) P. Solution remains clear	Benedict negative.
(f) G. Considerable green precipitate throughout indicating trace	Benedict trace.
(g) S. Heavy green precipitate after 30 seconds, turning yellow on further boiling	Benedict similar.

These tests indicate that in no case where a negative result was obtained with the new reagent, did Benedict's reagent give a positive reaction. In cer-

tain instances of diabetic specimens where Benedict's gave a negative result, slight traces were discovered by the use of the modified reagent. Test made on urinary specimens of normal individuals yielded negative results in every case.

An explanation of the manner in which the figures on the graduated tube are arrived at is now presented. The graduated tube (Fig. 2) has been *divided* into tenths of a cubic centimeter, but in order to save calculation, the figures have been made to *read off* the answer directly in per cent, provided *one c.c.* of the specimen is used for analysis. The manner in which these numbers are obtained is as follows:

$$\% = \frac{\text{mg. of glucose present}}{\text{wt. of specimen in mg.}} \text{ times } 100 \qquad (1)$$

The numerator of this fraction is equal to the amount of $CuSO_4$ started with, less the amount found unreduced, both expressed in terms of mg. of glucose. The amount started with is always the same and is equal to 50 mg. of glucose. The amount left unreduced is equivalent to the number of c.c. to which the filtrate has to be diluted in order to be of the same intensity as that of the standard with which it is being compared, multiplied by the value in mg. of glucose of 1 c.c. of that standard.

The denominator will be 1000, assuming 1 c.c. of urine is used. For other quantities this number will be different.

The right-hand side of the equation therefore becomes

$$\frac{50 - (\text{number of c.c., times the value of 1 c.c.})}{10} \qquad (2)$$

Putting for the value of 1 c.c. of the standard the letters $K\hat{a}$ $K\hat{b}$ $K\hat{c}$ for standards A, B and C, respectively, we may write instead of equation 2,

$$\frac{50 - (\text{number of c.c., times } K\hat{a}, K\hat{b}, \text{ or } K\hat{c})}{10} \qquad (3)$$

and since Standard A contains copper sulphate equivalent to 50 mg. of glucose in 25 c.c., 1 c.c. is equivalent to 2 mg. Similarly $K\hat{b}$ is 1 and $K\hat{c}$ is 1½.

If the unreduced copper sulphate had to be diluted, for example, to 20 c.c. in order to match Standard A, then the answer in per cent is according to equation 3,

$$\frac{50 - (20 \text{ times } 2)}{10} = 1.0$$

and 1 per cent, therefore, appears where the tube measures 20 c.c. If Standard B were being used for comparison, the $K\hat{b}$ being 1, the equation would read

$$\frac{50 - (20 \text{ times } 1)}{10} = 3.0$$

We find, therefore, in the right-hand column where the figures for Standard B appear, the figure 3.0 at the point where the tube measures 20 c.c.

Since the tube measures 25 c.c. at the point marked 0.0% it may be substituted for a 25 c.c. volumetric flask in making up the standards.

TABLE SHOWING RESULTS OF COMPARATIVE ANALYSES

AUTHOR'S METHOD	PAVY-KUMAGAWA METHOD	AUTHOR'S METHOD	PAVY-KUMAGAWA METHOD	AUTHOR'S METHOD	PAVY-KUMAGAWA METHOD
2.79		0.40	0.50	0.92	
2.72	2.76			0.92	0.93
		1.76	1.84		
1.48				1.04	
1.44	1.27	0.62	0.58	1.14	1.06
0.48		8.40	8.57	1.34	
0.45	0.49			1.40	1.47
		0.88	0.72		
1.56				0.40	
1.52	1.45	1.20	1.32	0.44	0.53
0.72		1.14	1.27	1.36	
0.74	0.67			1.34	1.27
		1.12	1.09		
0.98				0.20	
1.00	0.86	6.70	6.70	0.27	0.32
0.22		3.30	3.30	0.36	
0.21	0.23			0.40	0.33
		2.10	2.12		
1.20				0.47	
1.20	1.14	1.62	1.64	0.53	0.47
0.48		1.94	2.17	0.24	
0.48	0.45			0.26	0.24
		0.20	0.28		
0.22				2.10	2.00*
0.21	0.23	0.44	0.52	2.00	2.08
0.57		0.44	0.53	1.08	
0.60	0.57			1.02	1.03*
		1.14	1.17		
0.23				1.36	
0.19	0.20	5.5	5.8	1.44	1.35*
0.22		2.61	2.46	1.92	1.88
0.22	0.21	1.00	1.08		
				0.18	0.25
1.80		0.29	0.35		
1.78	1.88	1.26	1.27	1.80	1.88
0.70		1.98	1.91	2.61	2.76
0.67	0.73			0.90	0.87
		1.78			
1.44		1.72	1.82	1.74	1.92
1.40	1.85				
		0.48		1.00	0.99
0.68		0.45	0.42		
0.71	0.74			1.04	1.05
		2.60			
1.26	1.37	2.57	2.45	1.42	1.50
		1.18		1.34	1.40
0.21	0.22	1.22	1.29		
				0.42	0.53
6.5	6.72	2.20			
		2.24	2.16	1.42	1.35
1.0	0.86				

*According to Benedict's quantitative procedure.

SERUM VEAL AGAR: A DEPENDABLE SUBSTITUTE FOR ASCITIC OR BLOOD AGAR*

By N. S. Ferry, M.D., and Arlyle Noble, A.B., Detroit, Mich.

FOR reasons obvious to those interested in the routine culture of a large number of organisms, and especially mass growths, it was necessary to find, if possible, a substitute for ascitic or blood agar. Consequently a large number of different media were tested and it was determined that a veal agar (neutral to phenolphthalein) to which had been added normal horse serum (or any other serum) would successfully accomplish the purpose.

It was found, also, that the organisms would grow, in most instances, as well, if the peptone were omitted from the formula. This is an exceedingly practical point, especially where large quantities of peptone are consumed. (See "Peptone-free Media for Routine Culture Work," page 298 of this issue.)

FORMULA FOR SERUM VEAL AGAR

1. To 500 grams of lean chopped veal add 1,000 c.c. water. Macerate and allow to stand in refrigerator 24 hours. Strain through cheesecloth and bring to boil.

2. Filter, add 20 grams peptone (this may be omitted), 5 grams NaCl, and 30 grams finely chopped agar-agar.

3. Boil and adjust reaction to the neutral point with phenolphthalein (the success of the results with the medium depends in a large measure upon this point).

4. Filter and pour into test tubes, 3 c.c. each.

5. Sterilize fractionally, cool to about 45° C. and add 2 c.c. of sterile normal horse serum to each tube. The preparation should be about three parts of agar to two parts of serum.

ORGANISMS TESTED

1. Diplococcus pneumoniæ, Types I, II and III, cultures of which had been kept on whole blood (rabbit) agar. The majority of these cultures had recently been isolated.

2. Streptococcus viridans and hemolyticus, cultures of which had been kept on whole blood agar.

3. Various strains of streptococci which had been cultured on ascitic agar. The majority of these were fresh cultures from tonsils.

The following charts will convey some idea of the results of the tests. The figures in the charts are to be interpreted as follows:

1 or 1+ = Normal growth and is to be considered first choice.
1− = Abundant growth, but not quite as good as on control media.
2 = Moderate growth.
3 = Poor or slight growth.

All readings were made after 24 hours at 37.5° C. The cultures were then

*From the Research Laboratory, Parke, Davis & Co., Detroit, Mich.

	Growth On														
	Whole blood agar					Ascitic agar					Serum veal agar				
Number of generation after experiment starts	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th
Number of days in ice chest between transplants	4	4	6	14	17	4	4	6	14	17	4	4	6	14	17
Pneumococcus No. Type															
3 I	1	1	1	1	1	3	2	3	–		2	2	1	1	2
4 III	1	1	1	1	1	3	2	1	–		1	1	1	1	1–
5 I	1	1	1	1	1	2	1	1	3	2	1	1	1	2	1
6 II	1	1	1	1	1	3	1	2	2	2	2	1	1	1	1
7 III	1	1	1	1	1	2	2	2	2	2	1	1	1	1	1
8 IV	1	1	1	1	1	3	1	2	1	1	1	1	1	1	1
9 III	1	1	1	1	1	3	1	1	–		1	1	1	1	1
10 I	1	1	2	–		3	3	3	–		3	2	1	2	2
11 II	1	1	1	3	2	3	3	2	–		2	1	1	2	2
12 III	1	1	1	1	1	2	1	2	1	3	1	1	1	1	1
13 I	1	1	1	2	1	3	3	3	–		2	1	1	1	2
15 II	1	1	1	1	1	2	2	1	3	2	1	1	1	1	1
16 I	1	1	1	3	3	3	2	2	–		2	1	1	1	2
17 I	1	1	1	–		3	3	3	–		3	2	1	2	2
18 I	1	1	3	–		3	3	2	–		3	2	1	2	2
19 I	1	1	3	–		3	3	3	–		3	2	1	2	2

	Growth On											
	Ascitic agar						Serum veal agar					
Number of generation after experiment starts	1st	2nd	3rd	4th	5th		1st	2nd	3rd	4th	5th	
Number of days in ice chest between transplants	6	6	14	14	14	21	6	6	14	14	14	21
Streptococcus from tonsils												
No. 1 (a)	1	1	1	1	1	1	2	2	1	1–	1	1
1 (b)	1	1	1	1	1	1	2	1	1–	1–	1	2
3 (a)	1	1	1	1	1	1	1	1	1	1	1	1
3 (b)	1	1	1	2	1	1	1	1	1	1	1	1
13	1	1	1	2	1	1	1	1	1	1	1+	1
14	1	1	1	1	1	1	2	1	1	1–	1+	1
15	1	1	1	1	1	1–	1	1	1	1+	1+	1
22	1	1	1	1	1	1	1	2	1	1	1	1
33	1	1	1	1	1	1	1	1	1	1	1	1
43	1	2	1	2	1	3	2	1	3	2	1	1
45	1	1	1	1	1	1	2	1	2	1–	1	1
56 (a)	1	1	1	1	1	1	2	1	1	2	1	1

placed in the refrigerator, as has been the routine custom for ascitic or blood agar cultures, for the time indicated on the charts, ranging from four to seventeen days.

	Growth On															
	Whole blood agar						Ascitic agar						Serum veal agar					
Number of generation after experiment starts	1st	2nd	3rd	4th	5th		1st	2nd	3rd	4th	5th		1st	2nd	3rd	4th	5th	
Number of days in ice chest between transplants	14	6	14	14	14	21	14	6	14	14	14	21	14	6	14	14	14	21
Streptococcus viridans																		
No. 1	1	1	1	1		1	1	2	1	1		3	1	1	1	1		1
2	1	1	1	1		1	1	1	2	1		1	1	1	1	1		1
3	1	1	1	1		1	1	3	3	1		1	1	1	1	1		–
4	1	1	1	1		2	3	1	3	–			3	1	1	1		1–
5	1	1	1	1		1	1	1	2	1		1–	1	1	1	1		1
6	1	1	1	1		1	2	2	2	2		3	2	1	1	1		3
7	1	1	1	1		1	1	2	2	1		3	1	1	1	1		1–
8	1	1	1	1		1–	1	3	1	–			1	1	1	1		–
9	1	1	2	1		3	2	3	2	–			1	1	2	1		1–
10	1	1	1	1		1	2	3	3	2		–	1	2	1	1		3
11	1	1	1	1		3	1	2	3	1		–	1	1	3	1		3
12	1	1	1	1		2	2	1	2	2		–	2	1	1	1		3
Streptococcus hemolyticus																		
No. 1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1+	1	1
2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	1	2	2
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1	3	2	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	1–	1	1
10	1	1	1	1	1	1	1	1	2	1	1–	1–	1	1	1	1	1	1
11	2	1	1	2	2	3	3	2	3	–			2	1	1	–		
12	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

CONCLUSIONS

1. Cultures of diplococcus pneumoniæ grow as well on serum veal agar, after the second generation, as on whole blood agar and far better than on ascitic agar. These cultures lived longer on serum veal agar at refrigerator temperature, without transplanting, than on whole blood agar or ascitic agar. After two weeks four out of sixteen cultures on whole blood agar and ten out of sixteen on ascitic agar were dead, while all on serum veal agar were alive after seventeen days, and grew well when transplanted on the same media.

2. Cultures of streptococci grew as well and lived as long on serum veal agar as on whole blood agar and better than on ascitic agar.

3. Serum veal agar (veal agar, 2 per cent peptone, neutral, plus normal horse serum) can be used for maintaining cultures of most organisms ordinarily kept on ascitic or whole blood agar.

PEPTONE-FREE MEDIA FOR ROUTINE CULTURE WORK*

By N. S. Ferry, M.D., and Arlyle Noble, A.B., Detroit, Mich.

As a result of some experiments which necessitated the use of culture media prepared without peptone, it was found that the organisms under observation grew as luxuriantly as on standard media, suggesting the possibility of employing such media for routine purposes. It was determined, therefore, to give the method a comprehensive trial, as it involved a question of practical nature, especially to laboratories where large quantities of media are consumed daily.

Ten different kinds of media were prepared without peptone, part of them neutralized and part made 1.0 per cent acid, phenolphthalein being used as the indicator.

1. Liebig's Ext. bouillon—without peptone—neutral.
2. " " " " —1.0% acid.
3. Beef bouillon " " —neutral.
4. " " " " —1.0% acid.
5. " agar " " —neutral.
6. " " " " —1.0% acid.
7. Veal bouillon " " —neutral.
8. " " " " —1.0% acid.
9. " agar " " —neutral.
10. " " " " —1.0% acid.

A large variety of organisms was grown on the above media, culturing for several generations both freshly isolated strains and strains of various ages. For comparison, the same strains were grown on the standard media with Witte's peptone.

A tabulation of some of these results is given.

The figures in the charts are to be interpreted as follows:

1+ = Better than normal (on control media).
1 = Normal, abundant growth; first choice (except for an occasional 1+).
1- = Abundant growth, but not quite normal; second choice.
2+ = Abundant growth; third choice.
2 and 2- = Moderate growth.
3 = Poor growth.
4 = Very slight growth.

When two or more culture media are represented by the same figure, it indicates that there was no choice between them.

All results were read at the end of twenty-four hours. With each organism several generations were watched, not only in the incubator, but also at 5° C., so that the conclusions were not arrived at after a single generation at 37° C.

*From the Research Department, Parke, Davis & Co., Detroit, Mich.

Growth In Bouillon

Organisms	Media with Witte's Peptone			Media without Peptone													
	Plain Bouillon			*Liebig's Ext. Bouillon*		*Veal Bouillon*						*Beef Bouillon*					
	1.0% Acid			Neutral	1.0% Acid	Neutral			1.0% Acid			Neutral			1.0% Acid		
	1st gen.	2nd	4th	1st gen.	1st	1st gen.	2nd	4th	1st	2nd	4th	1st	2nd	4th	1st	2nd	4th
Staphylococcus aureus	1	1	1	3	4	1	1	1	2_+	1	1	1−	1−	1−	2_+	1−	1−
Staphylococcus citreus	1	1−	1−	3	4	1	1	1	2_+	2_+	2_+	1−	1	1−	2_+	1−	2_+
Staphylococcus albus	1	2−	3	2_+	−	1	2_+	1−	1	1−	1	1−	2−	2	1−	1	2−
B. pyocyaneus	1	1	1	2_+	−	1	2_+	1−	1−	1−	1	1−	2_+	1−	1−	1−	2_+
B. coli	1	1	1	3	−	1	1	1	1−	1−	1	1−	1−	1−	1−	1−	1−
B. paracoli	1	1	1	3	4	1	1−	1	2_+	1	1	1−	2_+	1−	2_+	2_+	2_+
B. typhosus	1	1	1	3	4	1_+	1_+	1_+	2_+	2_+	1	1	1	1	2_+	2_+	2_+
B. paratyphosus A	1	1	1−	3	4	1	1	1	2_+	2_+	2_+	1−	1−	2_+	2_+	2_+	2_+
B. paratyphosus B	1	1	1	3	4	1−	1	1	2_+	1−	2_+	1−	1	1−	2_+	1−	2_+
Streptococcus	1−	1−	1	4	4	1_+	1_+	1_+	2_+	1−	1	1−	2_+	1−	1−	2_+	1−

1st generation planted from 24-hour growths on agar into the above bouillons.
2nd transplanted from the 24-hour bouillon growths.
4th transplanted from third generation after being grown 24 hours and then placed in ice chest for 48 hours.

Growth on Agar

Organisms	Media with Witte's Peptone			Media without Peptone													
	Plain Agar			*Veal Agar*						*Beef Agar*							
	1.0% Acid			Neutral			1.0% Acid			Neutral			1.0% Acid				
	1st gen.	2nd	4th	1st	2nd	4th	1st	2nd	4th	1st	2nd	4th	1st	2nd	4th		
Staphylococcus aureus	1	1	1	1	1	1	1−	1	1−	1	1	1−	1	1	1−		
Staphylococcus citreus	1	1	1	1	1	1	1−	1	1−	1	1	1	1	1	1		
Staphylococcus albus	1	1	1	1	1	1	1−	1−	1−	1	1	1−	1	1−	1−		
B. pyocyaneus	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		
B. coli	1	1	1	1	1	1	1	1−	1−	1	1	1	1	1	1		
B. paracoli	1	1	1	1	1	1	1−	1	1	1	1	1	1	1	1		
B. typhosus	1	1	1	1	1	1	1−	1−	1	1	1	1	1	1	1		
B. paratyphosus A	1	1	1	1	1	1	1	1−	1	1	1	1−	1	1−	1−		
B. paratyphosus B	1	1	1	1	1	1	1−	1−	1	1	1	1	1	1	1		
	Ascitic Agar																
Streptococcus	1	1	1	1−	1	1	3	2_+	1−	2_+	1−	1−	1−	2_+	1−		
	Loeffler's Blood Serum																
B. diphtheriae	1	1	1−	1	1	1	2_+	2	2_+	1−	1−	2_+	1−	1−	2_+		

1st generation transplanted from 24-hour growths on plain, ascitic and Loeffler's Blood serum agar.
2nd generation transplanted from first after 24 hours.
4th generation transplanted from third after being grown 24 hours and then placed in ice chest for 6 days.

CONCLUSIONS

1. On peptone-free agar all cultures which are ordinarily grown on standard plain agar did so well that it was difficult to choose.

2. For the majority of cultures, veal broth media gave better results than beef broth, both with and without agar.

3. Both veal and beef broth gave far better results than Liebig's beef extract.

4. Throughout the whole experiment the neutral media gave better results than the 1.0 per cent acid.

5. Therefore, for organisms which grow readily on standard plain agar, neutral veal or beef media, without peptone, can be substituted.

A SIMPLE TEST FOR GLYCURONATES IN THE URINE

By Fritz C. Askenstedt, M.D., Louisville, Ky.

As a result of the activities of the intestinal flora a number of toxic products of the aromatic series, such as indol, skatol, cresol, and phenol, are to be found in greater or lesser amounts in the human intestine. Much of these products is eliminated with the feces, while a part becomes absorbed into the portal circulation. The toxic action of these aromatic bodies is neutralized by conjugation with sulphuric acid or with glycuronic acid in the liver. Thus are formed, on the one hand, indoxyl-glycuronic acid, skatoxyl-glycuronic acid, cresol-glycuronic acid, phenol-glycuronic acid; and, on the other, corresponding combinations with sulphuric acid, the so-called ethereal sulphates. That a part of these absorbed basic substances is destroyed by oxidization seems probable, another part undergoes different chemical transformations (hippuric acid, indolacetic acid, etc.), but the largest portion is excreted by the kidneys as above ethereal sulphates and glycuronates.

Except in cases of nephritis, indicanuria affords a fair index to the absorption of indol, which is formed from tryptophan mainly in the lower small intestine, but it bears no direct relation to the absorption of phenol, whose formation from tyrosin in the colon is usually slightly in excess of that of indol. While, as a rule, an excessive indol formation is attended by an excessive production of all the other above mentioned putrefactive products, it will readily be seen that owing to a difference in the character of the protein ingested, and possibly to a difference in the location of an intestinal lesion impeding peristaltic propulsion, the quantitative relation between the indol and the phenol productions may present considerable variations. As evidence of intestinal putrefaction—barring cases of putrefactive abscesses—a urinary test that will indicate the absorbed quantity of these two or more benzene derivatives is, therefore, ordinarily of greater utility than the test which represents but one factor. For such a purpose Folin's test for ethereal sulphates stands unchallenged in scientific value, but its execution demands considerable time, technic, and equipment, which necessarily confines its use to the larger laboratories. The glycuronates, which are capable of intense color reactions, are better suited for simple clinical tests. After some years of experimentation with Goldschmiedt's and Clifford Mitchell's reagent, alpha-naphthol (Modern Urinology), I feel warranted

in presenting the following test for glycuronates, which combines simplicity with a fair degree of accuracy:

The urine to be examined is diluted with water until its specific gravity is reduced to 1.001. For example, if the urine has a sp. gr. of 1.015 (correction being made for temperature), dilute 1 c.c. of urine with 14 c.c. of water; if the sp. gr. is 1.021, dilute 1 c.c. with 20 c.c. of water. (Diabetic urine should be diluted until its urea content is 0.1 per cent.) To 10 c.c. of the diluted urine in a test tube add one or two drops of a 1 per cent solution of alpha-naphthol in glycerin (alcohol impairs the test), and then 10 c.c. hydrochloric acid, sp. gr. 1.19. Mix by turning the tube over once or twice. Then let the tube stand in a dark, cool place for about twelve hours, after which the reaction is noted in reflected light. This is best done by holding a white surface, as, for example, a white blotter, behind the tube. Normally the fluid will remain colorless or show a mere suggestion of blue. If glycuronates are present in excess, there will appear a proportionate blue color, tinged with red. The depth of the color may be denoted by the customary use of plus signs, medium blue, the deepest color observed in the above dilution, being designated with ++++. If greater accuracy of estimation is desired, a standard solution of indigo blue, containing some indigo red, in sulphuric acid, may be employed by dropping it into another test tube of the same size, containing 20 c.c. of water. The tube receiving the standard solution is turned over after each drop is added and comparison of the two tubes is made, this procedure being continued until the nearest likeness of color is obtained, when the number of drops added is recorded. In twenty-four hours, or later, a slight cloudiness, with fading of color, appears, ultimately forming a sediment.

This test proves less sensitive to contaminating gases in the laboratory than do the indigo tests for indican. The slow reaction is its greatest disadvantage. Heating the mixture destroys or seriously impairs the reaction. Diabetic urine responds normally to the test, except that the blue color assumes a somewhat muddy hue. When first stale the urine reacts more promptly to the test than when fresh, but since by the action of putrefactive bacteria glycuronic acid is decomposed, and since certain compounds, as menthol- or thymol-glycuronic acid have been found to undergo spontaneous disintegration, the urine should preferably be fresh when tested.

Pentose in the urine may possibly give a bluish reaction. As I have been unable to procure any form of pentose in the market for experimental purposes, I can not at present produce any evidence bearing on this question. However, since pentosuria is a rare condition, and acute cases can usually be avoided by having the patient abstain for a few days from fruit, fruit juices, and malt liquors in the diet, it can not seriously detract from the value of the test. Moreover, a difference in the behavior of glycuronates and that of pentoses in the orcin test* will reveal the presence of pentosuria when suspected.

The presence of nitrites in the urine will inhibit the reaction of glycuronates,

*The urine is mixed with an equal volume of concentrated hydrochloric acid, a few small crystals of orcin are added and the mixture is then heated. As soon as the fluid becomes green or bluish green, it is cooled until lukewarm and shaken with amyl alcohol, which will render it bluish green (positive reaction).

Solutions of glycuronates, unlike pentoses, do not react to the orcin test directly, but only after the glycuronates have been disintegrated by boiling with an acid and their reaction is, therefore, several minutes tardier. (Olof Hammarsten, Fysiologisk Kemi, ed. 2, 358, 359.)

but may be suspected by their giving to the test fluid, immediately upon adding the hydrochloric acid, a light yellow tint.

It must be kept well in mind that the production and excretion of glycuronates are greatly augmented by ingestion of the following drugs, the toxicity of which becomes neutralized by paring with glycuronic acid: chloral hydrate, camphor, turpentine, morphine, menthol, arsenic, acetanilide, antipyrin, benzol, chloroform, curare, hydroquinone, naphthalin, phenol, resorcin, salicylic acid, salol, sulphonal, trional, and thymol. These remedies should, therefore, not be employed before the urine is collected for examination. It seems probable that a comparison of the reaction for glycuronates with the result of a reliable indican test will afford a valuable indication in cases of suspected poisoning with any of the above drugs, but opportunities for a clinical demonstration of this suggestion have not been afforded me.

The statement made by Schmiedeberg that glycuronates are formed only when sulphur is no longer available to the liver for the production of ethereal sulphates, seems incorrect, for glycuronates can always be demonstrated in undiluted urine, even when indican is absent.

In estimating the clinical value of the test it must be remembered that the excretion of an unusual amount of glycuronates or indican may be consistent with health. So long as the liver is functionally adequate to neutralize the entire amount of the toxic materials brought to it by the portal vein, little or no harm results, but a constant overstimulation of any function of the body tends to ultimate insufficiency, and a constant excessive production of indican and glycuronates is a positive signal of present or approaching danger.

AN ANTIGEN FOR USE IN COMPLEMENT FIXATION IN TUBERCULOSIS*

By Moyer S. Fleisher, M.D., and George Ives, M.D., St. Louis, Mo.

IT is not our intention to report here on the clinical value of this antigen or to discuss the significance of the complement-fixation reaction in tuberculosis. We desire only to present this antigen and state some reasons for our belief that it has a definite value in the recognition of tuberculous infection. The method of preparing the antigen is as follows:

Tubercle bacilli are isolated and grown from sputum on Petroff's medium. When the cultures show an abundant growth, forming definite raised masses, the organisms are scraped from the surface of the medium with a heavy platinum wire. It takes from four to eight weeks for the growth to become sufficiently abundant. The tubes of media are plugged in the ordinary manner. Every few days a portion of a cubic centimeter of water is added to the tubes. In all cases a number of different strains of organisms are used in making one antigen, and the number has varied from four to ten strains.

*From the Department of Pathology and Bacteriology, St. Louis University School of Medicine, and the Clinical Laboratory of George Ives.

The organisms are placed in a sterile open Petri dish or watch glass and allowed to dry overnight in a bacteriologic incubator at 37° C. It is not necessary to keep the mass of tubercle bacilli free of contamination, but contamination should be avoided as much as possible. The partially dried bacteria are weighed and then placed in a large, sterile, clean mortar. They are ground for three to four hours, vigorously and constantly. From time to time during the grinding, a scalpel is used to scrape the bacterial mass from the sides of the mortar and from the pestle. A few drops or as much as 1 c.c. of sterile distilled water is added when necessary to keep the mass of the proper consistency. Addition of sufficient water to make a fluid mass should be strictly avoided.

After grinding the necessary length of time, 0.85 per cent sodium chloride solution is added little by little, and the grinding is continued until the bacteria or remains of the bacteria form an even suspension in the diluent. Sufficient of the sodium chloride solution is added to make a 0.5 per cent suspension of bacteria. To this there is added an amount of 5 per cent carbolic acid equal to one-tenth the volume of the sodium chloride solution.

The finished antigen is dense, grayish, and opaque. The bacterial bodies and suspended cell-remains settle to the bottom, if the suspension is allowed to stand. The supernatant fluid is very slightly opalescent. Before use the antigen should be shaken so that the solid parts are again brought into suspension.

In the making of the antigen the most important step is the grinding. It has been demonstrated that less than several hours of proper grinding gives an antigen which is worthless or at least too weak for use in the method of complement fixation which we have used. Possibly if the grinding is continued for a longer time than we recommend, a more efficient antigen may be obtained.

The considerations which underlie the first attempts to make this antigen are largely theoretic. It seemed from a review of the literature that of all antigens used previous to the middle of 1915, the bacillary emulsions or antigens similar to these had given the more favorable results, with the possible exception of Besredka's antigen. It further appeared that there exist in the tubercle bacillus several partial antigens;* that different constituents of the tubercle bacillus call forth different antibodies which appear in the blood of the tuberculous individual at varying periods and in varying quantities. In preparing an antigen for the complement-fixation test, one should therefore attempt to obtain in that antigen all the various antigenic constituents of the tubercle bacilli. Neither an extract nor a filtrate do in all probability contain all these antigens. Although living and morphologically unmodified tubercle bacilli do contain them, in complement-fixation reactions they are possibly prevented from union with their corresponding amboceptors of the patient's serum by the waxy capsule of the bacillus. By grinding we may liberate water soluble antigenic constituents. If the theoretical considerations upon which this antigen was first prepared are supported by experiment, it must be concluded that this antigen is not included in the classification of antigens given by Miller.[1] We hoped to prepare, and evidence given below seems to indicate that we did prepare, an antigen which is both a suspension and a watery extract of tubercle bacilli.

We know that parasitic organisms may lose their virulence, and change in

*For the present discussion, we accept the correctness of the views of Much and Deycke.

their biological characteristics in other ways when they are cultivated on artificial media for several generations. This change in function is probably indicative of a change in metabolism, and probably of chemical composition. If, therefore, one desires to use as an antigen an organism as nearly as possible identical with the bacteria acting in the human body, it is essential to use an organism freshly isolated from the human. The use of the freshly isolated tubercle bacillus grown on Petroff's medium represents the simplest means of realizing the desired end.

It appeared furthermore that in order to obtain an antigen containing substances differing as little as possible from those of the actively pathogenic organisms, it would be necessary to avoid subjecting the bacilli to any agent which might lessen or destroy their antigenic qualities. We know that coagulation such as may be effected by heat, and coagulation such as may be brought about by some germicides, changes, at least in a small degree, the reactive properties of most antigens. Because of this consideration, the organisms were not killed nor modified in any manner before they were used for grinding. The use of several strains of tubercle bacilli in making the antigen is, of course, logical from the point of view of the individuality of various strains of organisms.

We are quite willing to admit the possibility that these theoretic considerations, which determined the method of making this antigen, may not have the significance that we attach to them. Whether the use of freshly isolated organisms, the avoidance of changing the bacilli in a manner or degree other than that involved in our method, the use of several strains of organisms, or the use of the entire organism, are essential considerations in the production of the most suitable antigen, must be determined by further experiment.

The power of our antigen to fix complement in the presence of the sera of tuberculous individuals resides, not only in the entire antigen, but in both the clear supernatant fluid and in the suspended matter. In an experiment with a series of cases in which parallel tests were made using as antigens the supernatant fluid as one antigen and the entire antigen as the other, corresponding results were obtained. The supernatant fluid is a suitable antigen, but it is weaker than the entire antigen. This experiment in itself would seem to distinguish this antigen from the Miller[1] antigen which according to Corper[2] shows little or no antigenic properties in its fluid portion. While the first antigen made by this method was produced in February, 1916, and while it was tested out in a few cases at that time, it was not until late in that year that extensive work was taken up with this antigen by one of us (George Ives) and sufficient cases were collected to warrant a definite idea of its value.

We believe that one can obtain with this antigen results equal to those obtained with any of the antigens which have been proposed by others. A series of cases have already been reported,[3] and since the publication of this report, a large number of tests have been performed which yielded results closely paralleling those of the reported series.

Comparing our results and interpretation of the test, we find ourselves in close harmony with Bronfenbrenner[4] and McCaskey.[5] The close parallelism between the results of the complement-fixation test for tuberculosis and clinical findings which have been reported by Miller[1] and Craig[6] we believe may well

not exist when a delicate test is applied for the recognition of tuberculosis. The very nature of tuberculosis teaches us that such a parallelism can not exist when the test is applied to a large group of individuals. The most striking instance of this lack of parallelism between clinical and serologic findings occurs in syphilis. In our unpublished data we find that over 50 per cent of syphilitics with a positive Wassermann give a positive complement-fixation test for tuberculosis. Most of these are cases of latent syphilis, and very few of them present any symptoms suggestive of tuberculosis.

We believe that this antigen possesses two further merits which have not been claimed for other antigens. First, owing to the addition of carbolic acid, it is sterile and remains sterile; second, it is stable, changing little if at all in its antigenic titer over a long period of time. At least one sample of antigen gave excellent results when used over a year after its preparation, and none of several antigens have shown deterioration after considerable lapses of time.

There is one other consideration which may give further evidence of the value of this antigen. For the past several months in the performance of the complement-fixation test, we have altered our technic and now titrate both complement and amboceptor each time the test is performed. We use a method of titration based upon a suggestion of Williamson.[7] We use in the test 1.5 units of complement and two units of amboceptor. We use heated patient's serum. Possibly our technic is not so delicate as that of the majority of workers in this field. If this is true, and if it be proved that our results with the complement-fixation test are as reliable as the results of those who use a more delicate technic, then it would appear that we have used a more sensitive antigen.

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EDITORIALS

Goldberger's Studies of Pellagra

FOR some years the United States Public Health Service has carried on an investigation into the etiology and epidemiology of pellagra and this work has been done largely, at least recently, under the direction of Goldberger. When this investigator began his inquiries, he was impressed with the following facts so forcibly that they determined the direction of his investigations.

It is the unanimous testimony of all who have observed and written about this disease that there is no evidence that it is ever transmitted to nurses, physicians, or attendants. In Italy where the disease has long been known and where special institutions have been provided for pellagrins, such transmission is unknown or at least unrecorded. No precautions have been taken to avoid transmission and still it has not occurred. This is confirmed by those who have made observations in this country. This seems to be admitted by all, even those who still claim that it is of bacterial or protozoal origin. Siler and Nichols, who studied the outbreak in the Peoria State Hospital some years ago, stated that no nurse, attendant, or employee of the institution at any time showed any evidence of the disease. In the Georgia State Sanitarium, where the sanitary

conditions have never been the best and where many of the wards are infested with cockroaches and bedbugs, and where this disease frequently develops among the patients, there has never been a case among physicians, nurses, attendants, or employees. This holds good for other southern insane asylums, such as the South Carolina and Mississippi State Institutions.

Impressed by these facts, Goldberger wrote in 1914 as follows: "In considering the significance of the foregoing observations, it is to be recalled that at all of these institutions the ward personnel, nurses and attendants spend a considerable portion of the twenty-four hours on day or night duty in close association with the inmates; indeed, at many of these institutions for lack of separate building or special residence for the nurses, these live right in the ward with and of necessity under exactly the same conditions as the inmates. It is striking, therefore, that although many inmates develop pellagra after varying periods of institutional residence—some even after ten to twenty years—which would lead one to believe it to be due to exciting causes within the institution, yet nurses and attendants living under identical conditions appear uniformly to be immune. If pellagra be a communicable disease, why should there be this exemption of nurses and attendants? To the writer this peculiar exemption or immunity is inexplicable on the assumption that pellagra is communicable. Neither contact in any sense nor insect transmission is capable of explaining such a phenomenon, except on the assumption of an incubation or latent period extending over ten to twenty years. In support of such an assumption, there exists, so far as the writer knows, no satisfactory evidence. The explanation of the peculiar exemption under discussion may be found in the opinion of the writer in a difference in the diet of the two groups of residents. At some of the institutions there is a manifest difference in this regard; in others, none is apparent. The latter would seem to be a fatal objection to this explanation, but a moment's consideration will show that such is not necessarily the case. The writer from personal observation has found that although the nurses and attendants may apparently receive the same food, there is nevertheless a difference in that the nurses have the privilege, which they exercise, of selecting the best and the greatest variety for themselves. Moreover, it must not be overlooked that nurses and attendants have opportunities for supplementing their institutional dietary that the inmates as a rule have not."

Pellagra is a rural disease and one usually of poverty. In other words, pellagra is frequent among the rural poor and rare among the urban poor. This is a striking characteristic and evidently it has some meaning. There is great poverty among certain classes in all large cities and still pellagra is distinctly rural. Goldberger points out that the chief difference in the diets of the rural and urban poor lies largely in the greater sameness in the former and the greater variety in the latter. The urban poor may buy any day a penny's worth of fresh meat or milk while the rural poor can not buy these articles because they are not for sale in their community. It is therefore reasonable to conclude that variety in diet is desirable in order to prevent the development of pellagra. In other words, the disease is not due to a diet deficient in calories, but to one deficient in certain food principles.

On this point Goldberger writes: "With regard to the question of just

what in the dietary is responsible, the writer has no opinion to express. From a study of certain institutional dietaries, however, he has gained the impression that vegetables and cereals form a much greater proportion in them than they do in the dietaries of well-to-do people, that is, people who are not as a class subject to pellagra. The writer is satisfied that the consumption of corn or corn products is not essential to the production of pellagra, but this does not mean that corn, the best of corn, or corn products, however nutritious and however high in caloric value they may be, are not objectionable when forming of themselves or in combination with other cereals and with vegetables a large part of the diet of the individual. In view of the great uncertainty that exists as to the true cause of pellagra, it may not be amiss to suggest that pending the final solution of this problem it may be well to attempt to prevent the disease by improving the dietary of those among whom it seems most prevalent. In this direction, I would urge the reduction in cereals, vegetables, and canned foods that enter to so large an extent into the dietary of many of the people in the South, and an increase in the fresh animal food component, such as fresh meats, eggs, and milk."

At this point in his inquiry, Goldberger stated his conclusions as follows:

(a) Pellagra is not a communicable disease, but is of dietary origin.

(b) It is dependent upon some as yet undetermined fault in the diet in which the animal or leguminous protein component is disproportionately small and the nonleguminous vegetable component disproportionately large.

(c) Pellagra does not develop in those who consume a mixed, well-balanced, and varied diet, such, for example, as that furnished by the Government to the enlisted men of the Army and Navy.

In 1915, twelve convicts in the Missippi State Prison were offered pardons by the Governor on condition that they submit to an attempt to produce pellagra in them by dietary means. One of these developed a prostatitis and was eliminated from the squad. The eleven were kept on a pellagrous diet from February 4 to October 31, 1915. Six developed between September sixth and twenty-fourth, typical dermatitis, justifying a diagnosis of pellagra. The nervous and gastrointestinal symptoms were mild but distinct. The diet consisted wholly of vegetable foods poor in protein and with the exclusion of milk, legumes and fresh meat. The convicts who were on the ordinary prison diet showed no sign of the disease at any time, before, during, or after this experiment. This experiment seems to have demonstrated quite conclusively that pellagra may be induced in healthy men by diet. This test would have carried more weight could it have been made in a region where pellagra is not so prevalent, but since there had never been a case on the prison farm, we are inclined to accept it at full value. In our opinion, its importance is greatly increased by the observations to be detailed later.

Goldberger and Waring describe an observation made by them at two orphanages at Jackson, Mississippi, as follows: "The first of these two to be considered is spoken of as Orphanage M.J. Cases of pellagra had been recognized at this institution every spring for several years. During the spring and summer of 1914, up to September 15, 79 cases of the disease were observed in

children at this orphanage. Although several of these were known to have had pellagra on admission or had developed it a short time after admission, a number appeared to have developed the disease for the first time after considerable periods of residence at this institution. The factor or factors causing pellagra and favoring its recurrence seemed, therefore, to be operative at this institution.

"The second of the orphanages, which will be referred to as Orphanage B.J., is located about half a mile east of Orphanage M.J. Here, as at M.J., cases of pellagra had been recognized every spring for several years. The writers are informed by the superintendent that a condition which he can not distinguish from that now called pellagra has occurred every year among the children ever since his connection with the institution, a matter of some twelve or thirteen years. From this description it is believed that there can be but little doubt that pellagra has prevailed in this institution almost if not quite, since its foundation in 1897.

"During the spring and summer of 1914, up to September 15, there were observed among the children in this institution 130 cases of pellagra. As at M.J. some of these were in recent admission; a large proportion, however, occurred in long-time residents.

"There appears to be little, if any, reason to doubt that the factor or factors causing the disease and favoring its recurrence have been operative at this institution for many years.

"At both institutions the hygienic and sanitary conditions found left much to be desired. Both were much overcrowded. The drinking water supply at each is drawn from the public supply. One is equipped with a water carriage sewerage system connected with that of the city; the other has the unscreened surface privy type of sewage disposal, and incidentally, we found here a great deal of soil pollution. At the very outset, it was requested that no change be made in hygienic and sanitary conditions and it is believed that these have remained as they were found and as they have been for several years.

"Since about the middle of September 1914, the diet at both orphanages has in certain respects been supplemented by the Public Health Service. At both institutions a very decided increase was made in the proportion of the fresh animal and of the leguminous protein foods.

"The milk supply was greatly increased. Provision was made to give every child under twelve years a cup of about seven ounces of milk at least twice a day. Those under six had it three times a day. Until the spring of 1915, the milk used was all fresh, sweet milk. In April of this year buttermilk was added to the diet. This was served at first only on alternating days to those over twelve years of age; later, when a sufficient supply became available, it was served at the midday meal to all.

"Eggs, except in cooking or for the sick, had previously not entered into the diet of the children. The writers prescribed at least one egg at the morning meal for each child under twelve years of age. It had been the custom to serve the children with fresh meat but once a week. Under the writer's direction, it was increased to three or four times a week.

"Beans and peas, which had been conspicuous in the diet only during the

summer and fall, were made an important part of nearly every midday meal at all seasons.

"The carbohydrate component of the institution diet was also modified. The breakfast cereal was changed from grits to oatmeal, partly because it was believed to be an advantage to reduce the corn element and in part because it was believed that the oatmeal would favor the consumption of milk. The corn element, though much reduced, was not wholly excluded. Cornbread was allowed all children once a week and grits to those over twelve years of age once or twice a week. Cane syrup or molasses, which it had been customary to serve freely at two or three meals each day, was for some weeks entirely excluded, and later allowed in small amounts at only three or four evening meals a week. The object in this was to reduce the proportion of the carbohydrate element."

The results of these alterations in diet may be summed up as follows: At M.J., 67 cases of pellagra completed the first anniversary of their attacks with no recognizable recurrence, nor were there any evidences of the development of the disease among the 99 nonpellagrous inmates.

In B.J., of 130 cases, 105 completed the anniversary with recurrence recognizable in only one. Of 69 nonpellagrins, not one developed the disease. In other words, aside from admissions during the year, only one case of pellagra existed in the two institutions, while of the 172 who lived on the improved diet for one year, there was only one recurrence at the end of that time. In our opinion it is unfortunate that at the end of the year and with the close of the supervision of the food, observation and record were wholly discontinued.

The observation conducted at the Georgia Sanitarium is more valauble because longer continued. This is a large insane asylum for both whites and blacks. Two wards, one for each race and both female, have been under the modified diet from December 1914, up to the present time (November 1917). No case of this disease has developed among nonpellagrins in these wards and there has been no recurrence of the disease among the pellagrins. In other words, the disease has been eliminated from these wards, while new cases and recurrences have appeared in other wards. There seems no reason why pellagra should not be eliminated from the whole institution under improved diet. It should be understood, however, that simply supplying proper food is not enough. It must be seen that every inmate gets it and eats it. It has been necessary to feed some of the insane with a tube.

The time after the provision of better food before improvement is noticeable is widely variable. The first evidence of betterment may show in the stomatitis, dermatitis or enteritis, generally in the improved condition of the mouth. Marked diarrhea is not a contraindication to a more liberal diet. Marked improvement in the skin lesions is not usually in evidence until the lapse of at least three months but finally these lesions quite disappear.

If diet be an important factor in the etiology of pellagra and since this disease is much more prevalent in the South than in the North, the comparative diets among the working classes in the two sections should be studied. Sydenstricker of the Public Health Service is engaged in this study and published a preliminary paper in 1915. This paper is based on data supplied by an investigation made by the British Board of Trade into the cost of living in American

towns in 1909, and a similar study made by the Federal Bureau of Labor in 1901. Sydenstricker says that the former developed the fact that the food price level in southern towns, weighted according to actual consumption in wage earners' families, was above the average in the rest of the country, the southern towns being from two to nine per cent higher than New York City. "Prices of the cheaper cuts of beef and of milk were in nearly every instance higher in southern towns than in the North. The conclusions indicated by the available evidence and in accordance with the principles of family income and expenditure already referred to, are that the wage earner's family in the South is at a greater economic disadvantage than in the northern states and that there is a greater pressure exerted in favor of sacrifices in diet in order to maintain an otherwise comfortable standard of living." Not only is animal food higher in price in the South but such foods are less widely distributed and consequently less available to the working classes. "The Bureau of Labor's data for 1901 showed that southern wage earners' families consumed over 25 per cent less protein foods, over 50 per cent more fats, and nearly 10 per cent more carbohydrates than northern families."

"For the meats and other protein groups of foods the geographic differences in consumption are significant. In the northern states the average family was found to consume annually between 1,000 and 1,100 pounds of protein, while in the southern states the protein consumption averaged between 700 and 800 pounds. The southern family consumed nearly a pound a week less of fresh beef, nearly half as much milk, very much less of other meats, and hardly any salt beef as compared with northern families. For the fats and hydrocarbon group of foods, even more significant differences are shown. While families in northern states were found to consume larger quantities of butter, families in southern states consumed over 60 per cent more lard and nearly three times as much salt hog products."

Sydenstricker has more recently collected a large amount of data bearing on the dietaries of the cotton mill employees, in relation to the incidence of pellagra in the different mill villages and in pellagrous and nonpellagrous families. When these facts are tabulated, they should prove of great value.

—*V. C. V.*

The Control of Communicable Disease Among Our Soldiers

AT present, this is one of the many big problems that confront the nation and demand solution. So far as the Medical Department of the Army is concerned, it is the biggest problem. While the mortality in our camps during the past few months has not risen to the great height that it did in 1898, it is sufficient to cause anxiety and to demand that every effort possible be made to reduce it.

That failure to supply proper and adequate clothing early has been a most potent factor in the incidence of pneumonia among our soldiers certainly has been abundantly demonstrated. A man can be chilled occasionally and for short period of time without injury to his health, but when one is cold night and

day, even the most robust is liable to succumb to any infection that may present itself. The spread of infection in our tents and barracks has been favored by overcrowding. These untoward conditions are being removed. The Secretary of War finds that most of the soldiers have received in January the clothing which should have been provided at the latest early in October. The Surgeon-General asked in July for at least fifty square feet of floor space per man in tent and barrack. This request has at last been granted, and provision is being made for its supply. Detention camps for incoming soldiers, where they may be kept under observation before mingling with others, have been ordered. Furthermore, additional space and housing are to be available for the prolonged care of convalescents before their return to duty, for contacts, suspects, and carriers. These are wise provisions and will do much to reduce the prevalence of communicable diseases among our soldiers.

However, it will be necessary to go further than this before we reach the greatest possible accomplishment, and we should not be satisfied with less. In the transformation of civilians into soldiers, some lives must be lost, but it is certainly the intention and desire of the whole nation that this loss be reduced to a minimum. Up to the present time, infections have been most prevalent in southern camps, but the season for respiratory diseases in the northern states has not yet been reached. This generally falls in February and March, and, while no one wishes to prophesy evil, it is wholly within the range of probability that during these months the respiratory diseases will be markedly augmented in all the camps and cantonments in the United States.

It must be evident to all thinking men that the conservation of the health of the nation is of prime importance at all times, but especially so under the stress of war. Each individual incapacitated by disease among the civilian or the military population reduces national efficiency, not only so far as the efforts of this individual are concerned, but to the extent that the services of others are absorbed in his care. A sick soldier is, so long as he is ill, not only one less in the effective force, he can not drill or otherwise prepare himself for the fighting line, but he is a possible source from which infection may be spread to others, and his care occupies the attention and time of others. A sick civilian is one withdrawn for the time being from the great industrial army which must stand behind the fighting force, and his care also withdraws one or more from the same service. In short, and it needs no argument, this is a time when all preventable sickness should be prevented.

It must be recognized that in the preservation of the health of the nation, no sharp line can be drawn between civilians and soldiers. Civilians constitute the source of supply of soldiers, and soldiers are constantly mingling with and returning to the civilian population. The relation between these two bodies is one of fluidity and not solidity. Failure to see this and act accordingly may lead to disaster in both portions of our population. At present, civilians are constantly invading the camps in which the infection becomes intensified and is returned to the civilian population throughout the length and breadth of the country. The assembly of troops, whether it be by draft or by voluntary service, acts like a dragnet, bringing the widely scattered infections from city, village and farm, from every part of the compass and from every class and con-

dition of citizenship. The camp acts as an incubator in which the infections develop and through which they spread and increase in virulence. Furloughed and discharged soldiers distribute the infection incubated and intensified in the camps, and even those who are sent across the seas bear these infections to our troops in France. If we are to win this war with the least possible expenditure of life, we must control, or at least abate, the infection among our soldiers, and this can not be done without attention to the abatement of communicable diseases among the people as a whole.

Suppose we have an army of three million men. At the same time, we have a civilian population of ninety-seven million. These are indeed one population, mingling and mixing at all times; one being converted into the other and then returning to the original source. Common sense shows that it is not possible to stamp out disease among 3 per cent of our population while we neglect the source among 97 per cent.

The health organization of the country, both national and state, extending down to the locality, should be and must be, if our troops are to be most effective, under one and the same control. With this in view, the following recommendations are offered:

(1) The United States Public Health Service, with its personnel, functions, duties, properties including its hospitals, quarantine stations, etc., should be transferred to the Medical Department of the army, and placed within the jurisdiction and under the command of the Surgeon-General of the army during the continuance of the war.

(2) If the above recommendation can be put into effect, the Surgeon-General of the Army should assign to duty in each state a medical officer whose function should be to cooperate with the state health authorities in the control and abatement of communicable diseases among the entire population of the state, both civilian and military, both temporary and permanent residents.

(3) In case the first recommendation be put into effect, the Surgeon-General of the Army should station at such points in each state as he may think wise, and we suggest in the proportion of one to fifty thousand population, additional military health officers, who, under the direction of the state officer mentioned in number 2 shall cooperate with the local health authorities in their efforts to control and abate communicable diseases.

Fortunately, the first recommendation is already legally provided for. Section IV of an Act of 1902 reads as follows:

"That the President is authorized in his discretion to utilize the Public Health and Marine Hospital Service in times of threatened or actual war to such extent and in such manner as shall in his judgment promote the public interest, without, however, in any way impairing the efficiency of the service for the purpose for which the same was created and is maintained."

Under date of April 6, 1917, the President promulgated an order in accordance with the above mentioned provision in the law. It is only necessary for the Secretary of War to ask the Secretary of the Treasury to transfer, for the period of the war, the United States Public Health Service with all its functions, duties and properties, to the War Department.

The practical working of these regulations should be something like the following:

The local military health official should keep himself well posted on the communicable diseases in his district, and he should be vested with all authority necessary in the control of the same. He should have under his direct supervision and command, all new troops, both those volunteering and those drafted within his district. He should see that these men are not unnecessarily exposed to infection, and he should know to what infections and to what extent they have been exposed. From the time a young man is selected for the draft until he reaches the camp to which he is to be assigned, he should be under the direct charge, supervision and control of the local military health officer. When the squad from that vicinity is assembled, this official should either in person or by a deputy, conduct these soldiers to the camp to which they were assigned and turn them over to the medical officer of the camp with a full statement of all exposures to infection. When a soldier is furloughed from camp, he should be transferred to the command of the local health officer and should be under his charge, care, and control during the whole of the time of his furlough. The local military health officers should make immediate telephonic or telegraphic reports to the state officer, and in case the military camp or cantonment is located in that state, directly to the division surgeon.

—*V. C. V.*

Military Antituberculosis Program Perfected

PLANS for a complete program for the prevention of tuberculosis in the army have been perfected by the National Association for the Study and Prevention of Tuberculosis working in cooperation with the Surgeon-General, the Y. M. C. A., and other agencies. This, it is predicted, will put the impending second draft on a better health basis than the first. The program will include not only a follow-up for every man discharged on account of tuberculosis, but a thorough-going health educational campaign among the soldiers.

Prior to the first draft the National Association began to outline a preventive campaign. Owing to the magnitude of the task and the many practical delays in perfecting and applying the details of this scheme, the results were not as encouraging as might be expected. This was due to the fact that the report of names of men rejected by the draft on account of tuberculosis was inadequate, the slowness of the machinery in getting under way, and the many difficulties in determining the status of the men.

Inasmuch as these enlisted or drafted men do not become accepted soldiers until after their probationary period lasting from three to six months in the various services, the government assumes no responsibility for the after-care of those whose health breaks down during that period. Hence, this problem belongs to the civilian boards of health and the unofficial health organizations.

The National Association program falls into two main divisions: (a) follow-up work and (b) educational work. The first obstacle to the follow-up program was Section Eleven of the Selective Service Regulations regarding the

second draft which forbids giving a record of a man's condition to anyone except certain designated officials. The National Association officers, however, placed before the War Department the importance of this work and were influential in persuading them to open the records of rejected men to state and local boards of health throughout the country, through the United States Public Health Service and the Council of National Defense.

Inasmuch as the above section of the regulations does not apply to men dismissed from training camps after they have passed draft boards, the association arranged with the Surgeon-General and the division surgeons in camps to receive the names of all men thus dismissed. These lists are divided up by states and forwarded to state associations and state boards of health for follow-up work. Where men are referred to localities where there are not at present facilities for this follow-up work, the association will use its good offices to promote the establishing of such facilities.

In the meantime, the Medical Department of the Army has perfected its machinery for weeding out these tuberculosis cases. Every man passed by the draft board after going into camp is examined by the regimental surgeon, reexamined by a tuberculosis board, and then if suspected of tuberculosis, again examined by a tuberculosis expert. This follows a general policy mapped out and recommended by the association.

A large number of men have already been accepted into the service who were known to be tuberculous, many of them formerly inmates of tuberculosis sanatoria. Part of the association's work has been to get in touch with every tuberculosis sanatorium and dispensary in the country and compile lists of all recent male inmates of draft age, giving the history of their cases and whether or not it was known if they were in the army at present. Hundreds of such names have already been received. This data is forwarded to the training camps, the men are located and the results are reported back to the sources of information.

Furthermore, the association has sent a letter to all of its fifteen hundred local cooperating agencies giving the provisions of the second draft and urging that these agencies procure the names and addresses of all the men of military age in their section who are known to have tuberculosis; get in touch with these men and arm them with the necessary affidavits to prevent, if possible, their being passed by the draft board, and recommend to the local draft boards the names of the approved tuberculosis experts in their section.

The association is also cooperating with the Surgeon-General's office to aid the government in providing sanatoria for those men who have been discharged from the service on account of tuberculosis after their probationary period has expired. All full-fledged soldiers and sailors returned from France or other stations will be cared for as near to their own homes as possible in sanatoria accommodations provided by the government. The government intends to utilize so far as possible existing institutions.

From the United States Marine Corps the association has secured each month a report of men rejected for tuberculosis from all its recruiting stations, and these men will receive the regular follow-up attention.

From the second or educational division of the program it is hoped to de-

rive the greater ultimate good by the establishment of fundamental preventive measures among the well.

The association is interested in any kind of an educational campaign among the men in the various military camps that will tend to promote interest and information with regard to the control and prevention of communicable diseases, and toward the promotion of public and individual health in general. In the mobilization of such large numbers of men in various camps throughout the United States there have developed an unusual number of somewhat serious epidemics of colds, coughs, pneumonia, measles and various other respiratory and communicable diseases. That all of these diseases can be controlled by education and by the exercise of adequate public health measures has been clearly demonstrated in the civilian population throughout the United States. Most of these epidemics are spread through ignorance and carelessness. It is inevitable where large numbers of men from all walks of life and with all possible diseases and variations of physical habits are thrown together in somewhat uncomfortable and crowded living conditions, that there will be an immediate increase in the amount of sickness from communicable diseases. It must be obvious, however, to even the most superficial observer, that if these men can be taught to maintain a reasonable standard of personal hygiene and can be given a knowledge of the methods and principles of the control of communicable diseases a rapid diminution in the sickness rate will follow.

In cooperation with the Educational Committee of the National War Work Council of the Y. M. C. A., the association will furnish a number of stock lectures dealing with tuberculosis together with lantern slides to illustrate them. It will also arrange to put the educational secretaries of each of the camps in touch with public lectures in and around their respective camps. The association has requested the War Department to give careful consideration to the desirability of appointing one or more special officers detailed to lecture on tuberculosis and allied health subjects in all of the army camps throughout the country.

The association has prepared a special circular entitled, "Red Blood," giving in brief and attractive form a message to the soldier relative to personal fitness, a health "Don't Card;" and a Public Health Manual may also be distributed, the latter being a textbook of personal hygiene.

The association will also arrange to distribute through the departmental executives of the Y. M. C. A., a number of special tuberculosis exhibits known popularly as "The Parcel Post Exhibit." In connection with these moving picture films and lantern slides will be used.

The field secretary of the association, Dr. Pattison, is visiting the training camps and supervising this educational work.

—*Philip P. Jacobs.*

Reporting of Accidents from Local Anesthetics

THE Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association has undertaken a study of the accidents following the clinical use of local anesthetics, especially those fol-

lowing ordinary therapeutic doses. It is hoped that this study may lead to a better understanding of the cause of such accidents, and consequently to methods of avoiding them, or, at least, of treating them successfully when they occur.

It is becoming apparent that several of the local anesthetics, if not all of those in general use, are prone to cause death or symptoms of severe poisoning in a small percentage of those cases in which the dose used has been hitherto considered quite safe.

The infrequent occurence of these accidents and their production by relatively small doses point to a peculiar hypersensitiveness on the part of those in whom the accidents occur. The data necessary for a study of these accidents are at present wholly insufficient, especially since the symptoms described in most of the cases are quite different from those commonly observed in animals even after the administration of toxic, but not fatal, doses.

Such accidents are seldom reported in detail in the medical literature, partly because physicians and dentists fear that they may be held to blame should they report them, partly, perhaps, because they have failed to appreciate the importance of the matter from the standpoint of the protection of the public.

It is evident that a broader view should prevail, and that physicians should be informed regarding the conditions under which such accidents occur in order that they may be avoided. It is also evident that the best protection against such unjust accusations, and the best means of preventing such accidents consist in the publication of careful, detailed records when they have occurred, with the attending circumstances. These should be reported in the medical or dental journals when possible; but when, for any reason, this seems undesirable, a confidential report may be filed with Dr. R. A. Hatcher, 414 East Twenty-Sixth Street, New York City, who has been appointed by the committee to collect this information.

If desired, such reports will be considered strictly confidential so far as the name of the patient and that of the medical attendant are concerned, and such information will be used solely as a means of studying the problem of toxicity of this class of agents, unless permission is given to use the name.

All available facts, both public and private, should be included in these reports, but the following data are especially to be desired in those cases in which more detailed reports can not be made:

The age, sex, and general history of the patient should be given in as great detail as possible. The state of the nervous system appears to be of especial importance. The dosage employed should be stated as accurately as possible, also the concentration of the solution employed, the site of the injection (whether intramuscular, perineural or strictly subcutaneous), and whether applied to the mouth, nose, or other part of the body. The possibility of an injection having been made into a small vein during intramuscular injection or into the gums should be considered. In such cases the action begins almost at once, that is, within a few seconds.

The previous condition of the heart and respiration should be reported if possible; and, of course, the effects of the drug on the heart and respiration, as well as the duration of the symptoms, should be recorded. If antidotes are

employed, their nature and dosage should be stated, together with the character and time of appearance of the effects induced by the antidotes. It is important to state whether antidotes were administered orally, or by subcutaneous, intramuscular, or intravenous injection, and the concentration in which such antidotes were used.

While such detailed information, together with any other available data, are desirable, it is not to be understood that the inability to supply such details should prevent the publication of reports of poisoning, however meager the data, so long as accuracy is observed.

The committee urges on all anesthetists, surgeons, physicians, and dentists the making of such reports as a public duty; it asks that they read this appeal with especial attention to the character of observations desired.

TORALD SOLLMANN, CHAIRMAN
R. A. HATCHER, SPECIAL REFEREE
Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.

TO THE EDITOR:

In the January number of the JOURNAL OF LABORATORY AND CLINICAL MEDICINE, in my article "War Deafness and Its Prevention—A Report of Tests upon Eight Preventives," the chart on page 230 was inserted through error. It belongs with a later report which will be published in the March issue of the JOURNAL.

—Stacy R. Guild,
Ann Arbor, Mich.